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MODIFIED ATMOSPHERE PACKAGING AND ITS FEASIBILITY FOR MILITARY FEEDING SYSTEMS

by

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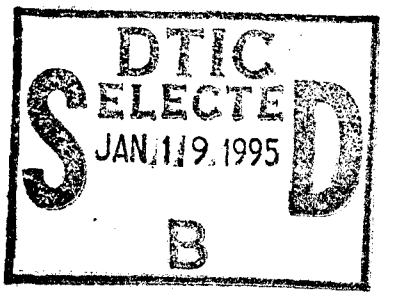

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The purpose of this project is to test the effects of different modified atmospheres and packaging systems on the shelf life extension of several foods, and their feasibility for military feeding systems. Several trials were conducted on packaging food products under modified atmospheres. Foods such as cooked boneless chicken breasts, hamburger patties and scrambled eggs were packaged under different gas mixtures, and with different packaging systems. There were also trials testing Time Temperature Indicators (TTIs), packaging equipment and local modified atmosphere packaging (MAP) producers.

These studies showed that a high barrier packaging is preferable for cooked meats. Initial gas analysis for some studies showed different gas levels than what was injected such as 20% less carbon dioxide (CO2), with the gas composition remaining quite stable thereafter. There is a need to determine if reduced carbon dioxide levels or gas mixture variation is due to the packaging equipment, namely, the form-fill-sealers or gas absorption by the food and to what extent. Microbial growth was erratic during most of the studies therefore microbial correlations were

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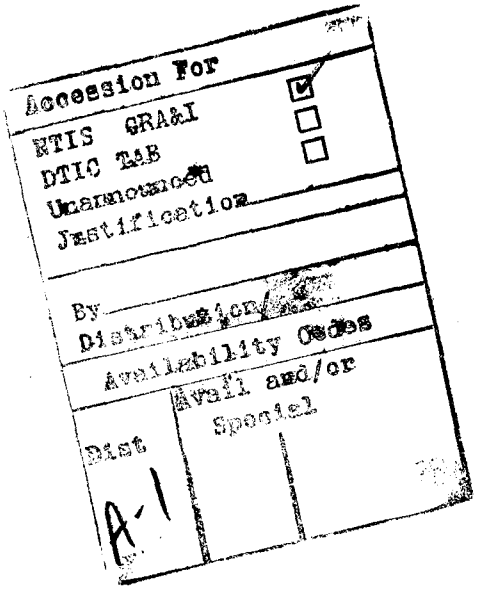
inconclusive. Strict Hazard Analysis Critical Control Point (HACCP) is needed for all MAP productions to limit microbial hazards. Sensory testing conducted on test samples was delayed by one week waiting for microbial results. There were no off flavors contributed by the gas mixtures. A more sensitive label than the TTI Lifeline label #76 was needed to detect short term temperature abuse and label #60 was identified as an efficient indicator for a 3-4 week expiration. There was indication that temperature abuse for 4 hours at 100°F was sufficient to cause harmful microbial growth in a MAP entree.

Due to the nature of MAP foods and their restrictive requirements, it is highly unlikely that many present day MAP products will be implemented in the military field ration system. MAP technology will be best used for oxygen and moisture control in shelf-stable rations, ethylene absorption in fresh produce and controlled atmosphere packaging (CAP) for shelf life extension during long distance transport of fresh fruits and vegetables within the military ration system.



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PREFACE

This technical report summarizes studies conducted by U.S. Army Natick RD&E Center to evaluate modified atmosphere packaging as a preservation technique for military rations or ration feeding. Part 3 describes the methods and procedures for six trials in the order that they were tested. Part 4 contains the results and discussions for these six trials. The List of Figures, and the information in Appendices A and B are arranged according to trial and not necessarily in the order cited in the text. This presentation was done for easy referencing. This effort was undertaken under Military Service Requirement 1532, AM 92-20, entitled Modified Atmosphere Packaging/Storage.

The authors gratefully acknowledge the contributions of Ms. Claire Lee, Dr. Anthony Sikes and Ms. Selene Watiwat for conducting the microbial tests, Ms. Margaret Robertson for conducting the gas and chemical analyses, Mr. Jay Jones for his assistance with the packaging material and equipment, Mr. Paul Dell for his assistance in conducting oxygen transmission analysis. Special thanks go to Ms. Michelle Richardson for her assistance and support.

MODIFIED ATMOSPHERE PACKAGING AND ITS FEASIBILITY FOR MILITARY FEEDING SYSTEMS

1. INTRODUCTION

1a. DESCRIPTION

Modified Atmosphere Packaging (MAP) is a preservation technique which can extend the refrigerated shelf life of minimally processed foods. MAP uses different mixtures of gases than are normally found in breathing air. MAP-packed foods have an extended shelf life because of the inhibitory effect carbon dioxide has on spoilage microbes [1]. A high barrier packaging material is commonly used for MAP systems to prevent any gas exchange with the outside atmosphere [2]. MAP foods are minimally processed, are not sterilized and are sensitive to microorganisms (i.e., they are not retorted in high barrier packages) therefore MAP products commonly need to be refrigerated or frozen.

Foods packaged in a modified atmosphere, controlled atmosphere, vacuum packed or sous vide (vacuum packed and slow cooked) are all included in the category of modified atmosphere packaging [3]. To a certain degree foods packaged with absorbing materials for oxygen or moisture are also considered to be modified atmosphere products. Some examples of the different types of MAP systems include the following:

1. Minimally processed foods are packed under modified atmospheres in high barrier containers or bags. This is done by the displacement of air in a package by another gas, by pulling a vacuum, gas flushing and sealing. The gas mixture may change with time depending on the type of food. Intermediate and high moisture foods must be stored in the refrigerator or freezer [4]. (Fig. 1a)

2. Controlled atmosphere packaging (CAP) is described as a preservation technique which establishes a specified gas mixture and maintains that environment. This can be accomplished by the use of specific permeable membranes or by maintaining a constant environment within a storage area using gas adjusting equipment [5].

3. Vacuum packaging removes all air from the package; high barrier bags are usually used. Vacuum packaging can be used for meats, cooked food and shelf-stable foods which may need refrigeration. (Fig. 1b)

4. Sous vide is a method of packaging raw foods under vacuum in high barrier films and then slow cooking. Sous vide is used for perishable gourmet foods which need refrigeration [6]. (Fig. 1c)

5. Oxygen absorbers and active packaging are used to modify the atmosphere within a food container after packaging. Oxygen absorbers reduce the oxygen level for foods packed in air or reduce residual oxygen levels of a specific gas mixture or vacuum packaged item. This system uses high barrier packaging with a reactive oxygen absorbing packet [7, 8]. Active packaging technology uses a reactive substance, which is incorporated into a layer of the packaging material or which has specific gas permeability. Active packaging with an oxygen-absorbing substance can reduce the oxygen level to 100 ppm, which will prevent mold growth and is

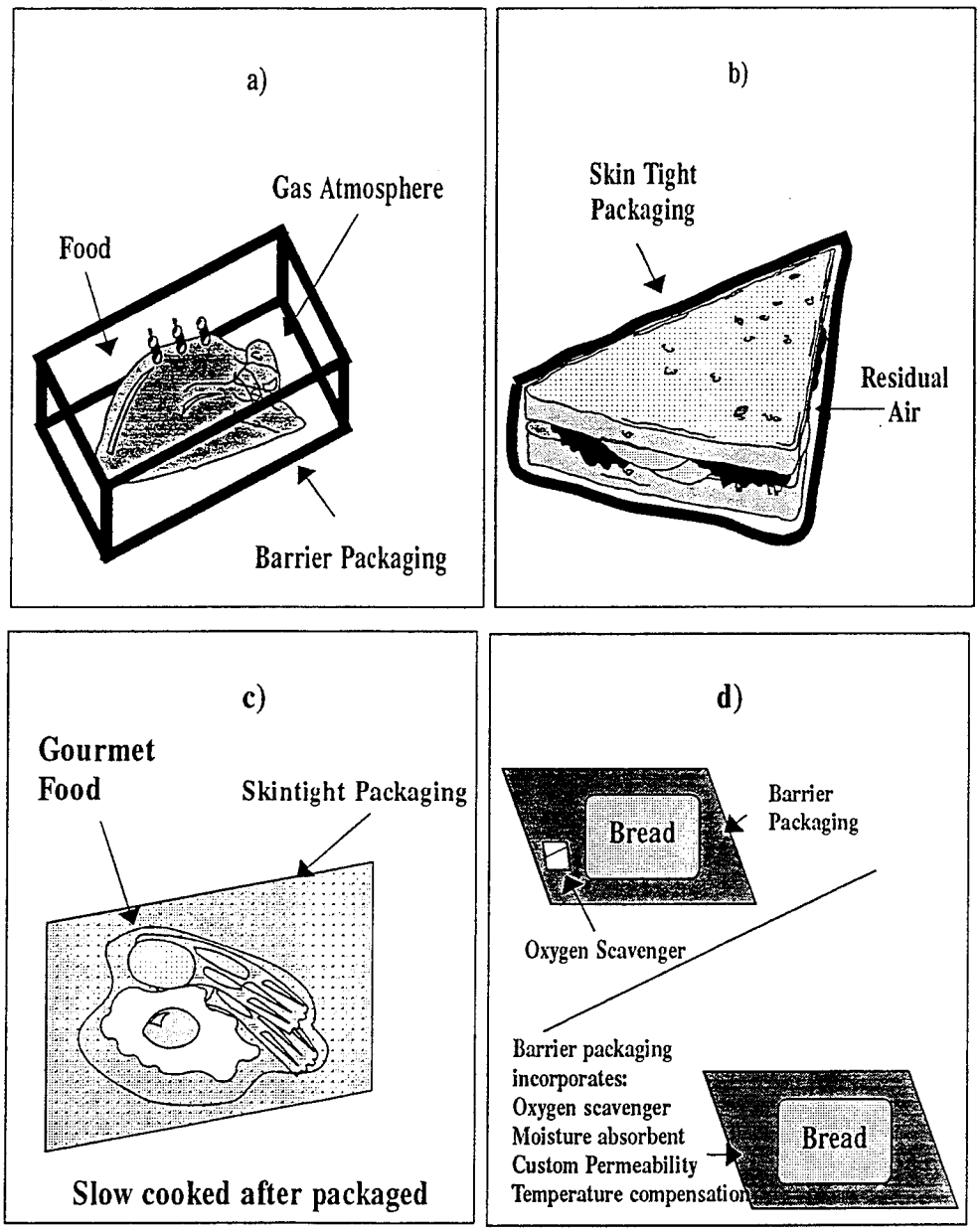

Figure 1. Types of modified atmosphere packaging systems essential components of a) modified atmosphere packaging; b) vacuum packaging; c) sous vide packaging; d) active packaging.

used mainly for starch or bread products at ambient temperatures. An active packaging with a gas-emitting substance will emit a customized gas mixture creating a modified atmosphere within the container after packaging [9]. Another type of active packaging is a packaging material with metal ions incorporated into the food contact layer, which will suppress or kill bacteria [10]. Yet another technique of modified atmosphere active packaging is a temperature compensating gas permeable packaging material, which is an efficient packaging used for whole or cut raw produce [11]. There is also a packaging system with specific gas permeability properties which can extend the shelf-life of fresh produce and meat to 30 days [12 - 14]. (Fig. 1d) Gas permeable labels applied to a container over vent holes to accommodate fresh produce respiration is an example of CAP which regulates the gas flow within the container [15].

The modified atmosphere packaged foods referred to within this report are of the type listed as 1a above. The gas mixture that surrounds the food is responsible for extending the shelf life by inhibiting microbial growth. MAP can extend the shelf-life of a perishable food by 400% [16]. MAP is being used to extend the shelf-life of baked goods, fresh fruits and vegetables, meats, and precooked ready-to-eat products without severe processing.

1b. FOOD TYPES

There are seemingly no limitations to the types of foods that can be packaged in a modified atmosphere. Foods can be put into two categories when considering MAP: 1) fresh produce or respiring living systems, and 2) nonrespiring or cooked foods. This is based on there being biochemical metabolic activity present to be respiring or no metabolic activity to be nonrespiring [17]. Critical factors, when considering MAP conditions, are the pH, Aw, the presence of preservatives and the respiration rate if it is a living system. The initial microbial load is also an important factor and the types of microbes expected to grow if given the right conditions [16]. The extent of processing and the type of food will affect these critical factors.

Designing MAP systems for fresh fruits and vegetables requires consideration of their respiration rate, the consumption of oxygen and the production of carbon dioxide [18, 19]. Reduced oxygen levels to $\leq$ 1% or elevated carbon dioxide levels to > 10% can retard the ripening and softening process, reduce respiration and ethylene production (produced by fruit or vegetable naturally to hasten ripening). Extended storage in low oxygen environment can also result in anaerobic respiration or metabolic damage. Ethylene absorption systems have been shown to maintain the quality of some light processed fruits and vegetables [20]. The ideal balance would be to minimize the respiration rate, which is different for each fruit and vegetable, without compromising the metabolic system. Permeable or slow breathing packaging material, which allows minimum gas exchange for minimum respiration, has been developed for fresh produce [21, 22].

When packing fresh red meat both the familiar red color and the microbial growth are factors to be considered. If red meat is exposed to a gas atmosphere without oxygen then oxymyoglobin is reduced to myoglobin. The pigment responsible for the bright red color, oxymyoglobin, will turn into a purplish color (i.e., reduced myoglobin). The optimum gas mixture to maintain the red color and microbial

suppression is between 85-90% oxygen and 10-15% carbon dioxide [23]. Reduced oxygen may reduce rancidity, but the longer the shelf life with reduced oxygen, the greater chances that color may be affected. The controlling factor to shelf life is oxygen but consumer acceptance will be greatly affected by the meat color. Elaborate two-phase packaging systems have been developed for MAP packaging fresh meat, which extend the shelf life during shipping with a low oxygen gas mixture and then allow the storekeepers to prepare product for consumer viewing. One packaging system for MAP meat consists of a high barrier dome lid over a gas permeable film. The high barrier dome holds a gas mixture, i.e., 30% carbon dioxide and 70% nitrogen, which will be removed just prior to consumer viewing. The gas permeable film will permit oxygen to restore the meat's red bloom in about 20 minutes [13, 14].

Cooked or minimally processed foods are nonrespiring systems. There is very little to no gas exchange between the food and its modified gas atmosphere except some absorption of carbon dioxide when a high barrier packaging is used. A common gas mixture used for nonrespiring foods is 75% carbon dioxide, 15% nitrogen and 10% oxygen [16].

1c. GAS MIXTURES

Gases commonly used for MAP are carbon dioxide, nitrogen, and oxygen but the concentrations should be tailored for individual food products. Carbon dioxide is the gas that effectively increases the lag phase and generation time of microbes and is responsible for inhibiting spoilage bacterial growth [24]. Carbon dioxide has a bacteriostatic effect and some bactericidal effects to inhibit the growth of S. aureus, Salmonella, Yersinia and gram-negative bacteria. It also acts as an insecticide and will inhibit mold growth. The mechanism is unknown but it may be due to a decreasing pH. The gaseous carbon dioxide dissolves with the aqueous portion of food, reacts with surface water to give carbonic acid and a lower pH [25]. It is also thought that carbon dioxide alters the bacterial cells' permeability and causes enzymatic inhibitions [23]. The inclusion of low levels of oxygen is a preventative measure to inhibit anaerobic growth if pathogenic growth is a concern [26]. If a processing operation is clean there may be little need for oxygen. Nitrogen is used as the inert balance, a filler and to lower the oxygen concentration.

There may be gas absorption by the food after packaging, which may cause packaging collapse. Many MAP manufacturers avoid package collapse by slight gas overfilling and within a few hours to a few days the gas/food equilibrate leaving the package at normal fill.

The amount of gas as compared to food is an important consideration when packaging systems are chosen. The proportions of gas to product can determine the effectiveness and how long that effectiveness will be at its optimum. The amount of surface area the product has exposed to the modified gases can also determine the systems' effectiveness. In both cases more is better, i.e., more gas to product and more surface area per product.

1d. PACKAGING

The function of the packaging is three fold. The first is to accommodate, contain (enclose or surround) and protect the integrity of the food item. Packaging systems for MAP can vary greatly from a

laminated cardboard or high barrier material, preformed containers or formed on line, rigid or semirigid container with a peelable or nonpeelable lid film to a high barrier pouch. The second is to provide protection against contamination during storage to maintain a high quality food with an extended shelf life and thirdly to contain and hold the modified gas atmosphere around the food. The more effective the barrier properties of the packaging material the better it will prevent any gas exchange between the outside and the packaging atmosphere [27].

1e. FOOD SAFETY, SANITATION AND HAZARD ANALYSIS CRITICAL CONTROL POINTS (HACCP)

A clean operation is of the utmost importance when processing foods that will not be sterilized during production, such as MAP foods. To assure food safety, precautions such as pasteurization must be taken in all food processing. There are special precautions for MAP foods because a MAP food will not be sterilized and contamination or mishandling at any point in production can make it unsafe for consumption. Common food spoilage microbes give off putrid odors and that is an indication that pathogenic organisms may also be present and that the food is unsafe to eat. Higher than normal carbon dioxide levels within a MAP product will suppress the growth of spoilage organisms [1].

The packaging process of MAP products is usually performed in a clean, air conditioned area. All personnel should be required to dress so as to not contaminate the product, at all critical control points. Critical control points are locations identified along the processing or production line where measures can be taken to prevent safety hazards. During formulation or processing, which can be quite elaborate, the minimization of contamination becomes critical to assuring the quality and safety of foods [28]. The strict monitoring and control of all processing steps is a technology unto itself and the guidelines to this system are called Hazard Analysis Critical Control Point (HACCP) [24]. At this time there are no regulations governing the processing of "new generation refrigerated foods," which include MAP foods. It is the sole responsibility of the manufacturers to assure the safety of their product.

2. REQUIREMENTS

2a. ARMY REQUIREMENTS

There is a need for a military ration that is familiar to the soldier and accepted as a home cooked meal would be accepted. Tray packs and MRE components that are retorted or freeze dried make up most of the existing shelf-stable military rations. It is not yet feasible to modify a freeze dehydrated or a retorted ration to approximate a fresh cooked meal. MAP's ability to extend the shelf life of a cooked refrigerated food is a good possible alternative. The initial military requirement called for meals with the fresh quality of an A Ration with a longer shelf life and with minimal on-site preparation for field feeding [24]. The army requirements for shelf-stable rations is maintained quality for three years at 80°F/ 27°C (DoD 4145.19-R Storage and Materials Handling, Ch.5 Storage of Special Commodities, Sect.5 Subsistence, 1991). A MAP food has the fresh quality of an A Ration and requires minimum on-site preparation but does

not fulfill the shelf life requirement. The shelf-life of a perishable that has been minimally processed and packed in MAP is between 8 and 24 weeks at refrigerated temperatures depending on the food category.

2b. STORAGE

MAP for perishable foods is not a substitute for proper refrigeration. Storage and distribution temperatures will affect the length of shelf-life as a function of time and relates to storage stability. A difference of a couple of degrees in temperature over time will significantly affect the storage stability [16]. Controls on storage temperature and distribution practices are important for quality. Temperature monitors or time temperature indicators (TTIs) are methods used to ensure indications of temperature abuse whether from excessive temperatures or storage time [30, 31].

2c. BENEFITS/DRAWBACKS

The major benefits to utilizing a MAP product are those of enjoying a high quality food, which has not been overprocessed. MAP items possess a superior quality, namely texture, as compared to an overly processed or retorted food product. MAP products require very little preparation time, simply heat in package (duo-ovenable), similar to the Tray Pack ration or remove from package and heat. Some packaging systems will allow microwaving.

In regard to fresh fruits and vegetables, MAP can reduce spoilage and quality loss and can increase the percentage and quality of produce that reaches the consumer [17]. MAP can expand the radius of distribution by extending the quality such as color, moisture, flavor and maturity retention of produce.

In the commercial sector MAP perishables have their own category called a 'new generation chilled food'. Some foods packed under MAP are shelf stable but most require refrigeration. This presents logistical challenges for storage and distribution for commercial users and, especially, for the military. When temperature abuse occurs, there may be a safety risk although time temperature indicators may be used to warn against health hazards. MAP requires quick and efficient distribution while maintaining refrigerated temperatures to ensure high quality. The microbial status of a MAP product is dependent on the starting ingredients, handling conditions, storage temperature and time, and distribution temperature and time.

3. METHODS AND PROCEDURES

3a. INTRODUCTION

Since there is no universal standard for safety of MAP products, we followed commercial practices whenever possible when producing a MAP product. The shelf stability of some locally purchased MAP products were evaluated. Several experiments were run to evaluate all aspects of MAP food. Foods such as boneless chicken breast, hamburger patties, and scrambled eggs were tested for optimum gas mixture, packaging systems, shelf life, acceptability, and quality. The experiments run were:

- Boneless chicken was packaged in large mason jars under different

gas mixtures and stored at refrigerated temperature for 5 weeks to test an in-house developed gas delivery system. Shelf-life quality was used to test optimum gas mixture and gas composition was tested during the test period.

- Boneless chicken was also packaged at a local MAP producer to test a form-fill-seal packaging system.

- Commercial MAP products with time temperature indicators were stored at refrigerated temperature for 4 weeks during which time some samples were subjected to temperature abuse for 4 hours at 70 or 100°F. The indicators were evaluated for their sensitivity to temperature abuse and the food for its quality during the testing period.

- Hamburger patties were packaged in trilaminated foil pouches under different premixed gas mixtures. The gas volumes, gas composition and microbial growth were evaluated initially and after 4 weeks storage at refrigerated temperatures.

- Scrambled eggs were MAP packaged on a form-fill-sealing machine using a thermoformed packaging system. The eggs were packed under a test gas mixture, or control air, stored at refrigerated temperatures for 6 weeks. Gas and microbial analyses were run during the testing period.

- Scrambled eggs were packaged at a local MAP producer and were tested for gas composition and microbial growth.

Gas compositions were analyzed by gas chromatography and Analysis of Variance (ANOVA) statistical analysis was calculated with probability levels of $p<0.05$ for Trials 1, 2, 4, 5 and 6. Microbiological analysis was also run using standard methods of the Official Methods of Analysis of the Association of Analytical Chemist (AOAC, 1990). The microbiological data from Trials 3 & 4 were converted to log values and ANOVA was calculated with probability levels of $p<0.05$. Oxygen transmission rates of packaging materials were measured using a MOCON (Minneapolis, MN) set at 1% relative humidity and 70°F. ANOVA was also run on the sensory scores for Trial 3 with probability levels of $p<0.05$. Reflectance data collected in Trial 3 were statistically analyzed by ANOVA with probability levels of $p<0.05$. The slope and correlation coefficients were also calculated from the reflectance data.

3b. Trial 1 : NATICK MAP CHICKEN

Chicken breasts were baked and packed in a modified atmosphere for the purpose of testing our in-house procedures. The in-house procedures include gas filling apparatus, glass mason jars with modified lids used for packaging, and anticipated Hazard Analysis Critical Control Points.

Raw, boneless, skinless, chicken breasts were washed under cool running water and placed onto sterile paper towels to remove excess water. The raw chicken breasts were then placed onto paper-lined sheet pans, covered with foil and baked (no oil or spices) at 350°F in a convection oven for 20 minutes. The foil was removed and the uncovered chicken was then cooked for an additional 10 minutes. The chicken was removed from the oven, covered with foil again and chilled in a -20°F blast freezer for 20 minutes.

Two to three chicken breasts weighing approximately 240-250 g raw and 165 g cooked each were put into sterile wide mouth quart mason jars and capped. The lids were modified with two silicone sealed rubber septums to allow insertion of large needles for vacuum, flushing and injecting gas mixtures. In total 75 jars of chicken were gas injected and sealed.

Heavy gauge needles 3.7 and 15.3 cm long were epoxied to stainless steel three-way stop cocks. The shorter needles were used for vacuum outlet and the longer needles were used for injection and flushing with gas mixture.

The gas filling apparatus consisted of gas tanks, with tubing connecting to the following: a gas blender, control valve, inlet septum needle, container, outlet septum needle, vacuum gauge, control valve, and vacuum pump. There were three gas tanks containing carbon dioxide, oxygen and nitrogen connected by tubing to a gas blender, which could be adjusted to regulate any gas mixture needed. Initially a vacuum was pulled for 30 seconds in an attempt to achieve 0 atm. A gas mixture was then injected for one minute followed by opening the outlet septum and flushing for a full three minutes. Five different gas mixtures were tested: Control Air, Gas 1 (40% / 2% / 58%), gas 2 (40% / 5% / 55%), Gas 3 (40% / 0% / 60%), and Gas 4 (60% / 0% / 40%) of carbon dioxide, oxygen and nitrogen, respectively. Each lid was dipped into melted paraffin for extra sealing precaution. Chicken samples were stored at refrigerated temperatures for 6 weeks. Gas levels were monitored throughout the study by gas chromatography analysis.

Preliminary tests included injecting gas mixtures into empty jars and analyzing the despatch to test the gas filling apparatus and the integrity of the modified glass container lids. Gas analysis on the samples was done initially and then on a weekly basis on the same three samples for each gas variable.

Microbiological analysis was conducted on the raw, cooked initial, and MAP packaged samples and included testing for aerobic and anaerobic microbes and for Escherichia coli and Salmonella. Microanalysis was conducted weekly for as long as the MAP-packaged samples were considered to be acceptable. Sensory evaluation was attempted but the lag time between sample withdrawal and microbiology test completion made it prohibitive and unreliable.

3c. Trial 2 : TRIO'S MAP CHICKEN (Trio's, Chelsea, MA)

A similar chicken test was conducted at Trio's, a local MAP producer of pasta and sauce products. Chicken breasts were prepared and handled in the same manner as described above, but the chilled chicken was then transported on ice to Trio's to be packaged. Trio's packaged 13 cooked chicken breasts in (40%/ 0.5%/ 60%), 7 chicken breasts in (40%/ 2.5%/ 60%), and 7 chicken breasts in (40%/ 0.35%/ 60%) carbon dioxide, oxygen and nitrogen, respectively. They were packed in a high barrier film (proprietary) using a T.W. Cutter horizontal form-fill-sealing machine. They were then stored at 40°F for 4 weeks.

Microbiological analysis was run after 2 weeks storage. The test included aerobic and anaerobic plate counts, Escherichia coli and Salmonella. Gas analysis for carbon dioxide, oxygen and nitrogen was done initially, and after 2, 3, and 4 weeks of storage.

3d. Trial 3 : EVALUATION OF TIME TEMPERATURE INDICATORS (TTIs) AND COMMERCIAL MAP ITEMS

Commercially procured MAP chicken and lasagna were stored at refrigerated temperatures with Time Temperature Indicators (TTIs) attached on the package lids. During a four week period some samples were exposed to abusive temperatures. All samples were tested for microbial growth,

acceptance, and reflectance change of indicator labels.

This test indicates the products' sensitivity to the exposure of higher temperatures for relatively short periods of time. This test will also show the relationship between microbial growth, acceptance, time and temperature, and label type and reflectance over an uneventful storage period.

The TTIs are self-adhesive labels, which include a bar code and a reflectance area (Fig. 2). The bar code includes a 10-digit identification number to differentiate each label and to aid in product/container itemization followed by a 2-digit, label type number. The reflectance area is a temperature sensitive stripe between two outer reference stripes. The temperature sensitive polymer stripe will darken and the reflectance will decrease at a predictable rate dependent on time and temperature showing the cumulative temperature exposure. Labels are kept at -58°F until needed to prevent thermal exposure. The reflectance data can be read/measured and stored with a hand-held computerized scanning device. The data can then be retrieved by computer using Life Lines Technology, Inc. data collection and acquisition software. This software is part of a monitoring system used for inventory management, quality control and shelf life evaluation [30, 31].

Sensory data were collected by a small group of in-house experienced panelists. The group was briefly trained for these products and instructions for future testing developed (B-3a) at the first session. All sessions thereafter consisted of a rating period followed by discussion. The sensory panel rated appearance, odor, flavor, texture and overall quality on a 9-point quality scale (government form STSNL Form 964) for both the lasagna and chicken (B-3b). The average for each attribute was used for statistical comparison. For the chicken the panel also rated characteristics using questions extracted from techniques used for sensory profiling of canned boned chicken [32].

Sixty-six single serve packages of Tyson's roasted drumsticks (3 drums/pkg) and 60 Deluca's meat lasagna (9-10 oz pkg) were procured by the case at the local grocery store. Both were packed in high barrier clear material, the chicken was vacuum packed and the lasagna was in a thermoformed container. The lasagna had a "use fresh or freeze by date of 25 days" and it was purchased 12 days after date of pack. The chicken had a code date of 19 days and it was purchased 4 days after date of pack.

Three label types, each of different sensitivities, were used for this study, (Life Lines Technology Inc. fresh scan indicator labels number 60, 67, and 76). Relatively sensitive labels were chosen for this study to pick up subtle temperature changes, such as during short periods of high temperature exposure, which may result in significant microbial growth. All three label types were attached to the inside of each case and onto two randomly chosen packages within each case. The samples were stored in a 35°F refrigerated box. Six initial scannings of each TTI label were read, averaged and stored as a baseline for comparison. Control samples were stored in the freezer.

Initial microbial and sensory analysis were also run. Microbial acceptability was required prior to sensory testing. Microbial analysis took approximately one week to perform leaving no alternative but to conduct sensory analysis one week past the planned withdrawal evaluation date. Microbial analysis was done by standard methods here at Natick. Samples were tested for aerobic plate count, yeasts and molds, and pH.

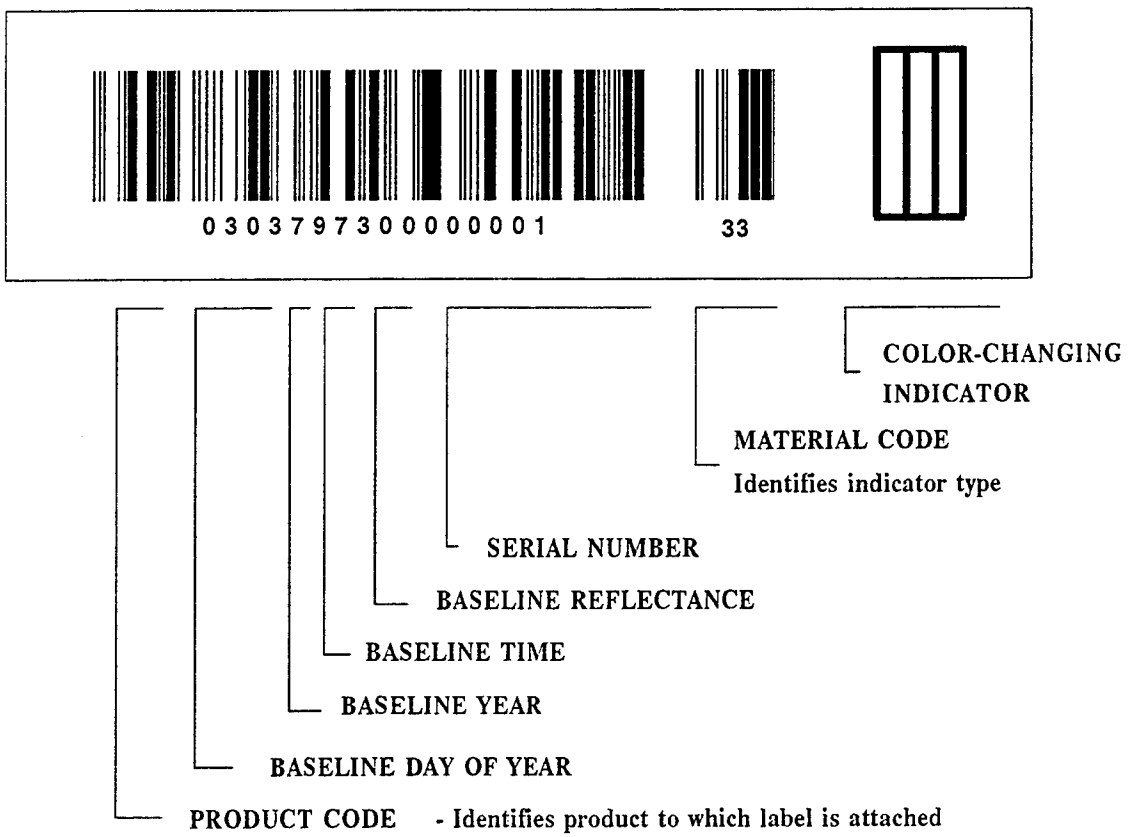

Figure 2. Time-Temperature Indicator label and the information it contains.

Following 1 week of storage, randomly chosen samples were removed from storage, exposed to temperatures of 70° or 100°F for 4 hours and then returned to refrigerated storage: the temperature exposure was registered by the TTI. Sensory testing was done on the control group (stored at -10°F for 1 week), 1 week group (sample held at 35°F for 1 week), and both abused groups (sample stored at 35°F for 1 week then exposed to 70°F for 4 hours, and sample stored at 35°F for 1 week then exposed to 100°F for 4 hours). Microbial analysis and TTI scanning were run also on the 1 week and both abused groups.

After 2 weeks of storage, sensory analysis was run on the control group, 2-week sample, and both abused groups which had been rechilled for 1 week. Microbial analysis and TTI scanning were also run on the 2 week and both abused groups.

After 3 weeks of storage, TTI scannings were run on the samples stored at refrigerated temperatures for 3 weeks.

After 4 weeks of storage, sensory analysis was run (appearance and odor only) on the 4-week sample, and both abused groups. The testing schedule is summarized in Table B-3d.

There will be three sets of data for the TTI scannings, uneventful storage for 3 weeks, abused at 70°F group, and abused at 100°F group.

3e. Trial 4 : NATICK MAP POUCH HAMBURGERS

Frozen beef patties were grilled, chilled and sealed in trilaminated pouches under control air and six test gas mixtures. Microbial and gas analysis as well as gas volume measurements were tested on random samples. This test was done to test the efficiency of using a pouch packaging system for MAP hamburgers.

Frozen beef patties were grilled for five minutes on each side until the center reached 130°F. Hamburgers were cooked in batches of 12 at a time. Grill was wiped clean and the cooks gloves were changed between batches. The excess grease was drained and hamburgers patted dry using previously sterile paper towels and aluminum foil. The paper towels were removed and the cooked beef patties covered with aluminum foil were blast frozen for approximately 10 minutes. They were then removed from the freezer into a refrigerator.

All precautions were taken to prevent contamination. Hair nets, gloves, masks, and lab coats were worn by all personnel while handling the beef patties. Paper towels, aluminum foil, utensils, plates, sheet pans, and trilaminated pouches along with any other items which came into contact with beef patties or used while handling the beef patties were pre-sterilized in the autoclave. Foot-long tubing with cotton stuffed into both ends along with filters and clamps was also autoclaved for sterilization and used for the gas connections between gas cylinders and a pouch sealing machine. Gas mixtures were purchased as premixed tanks and the sterile tubing was changed for each gas mixture. The gas mixtures tested were gas 1 (25% / 0% / 75%), gas 2 (75% / 0% / 25%), gas 3 (73% / 2% / 25%), gas 4 (70% / 5% / 25%), gas 5 (25% / 2% / 73%), gas 6 (97% / 3% / 0%) of carbon dioxide, oxygen, and nitrogen, respectively, and control air. The pouch sealing machine pulled a vacuum, then injected and flushed a premixed modified atmosphere while sealing beef patty in a trilaminate pouch. The Reiser pouch sealer was previously tested for gas filling settings, such as vacuum, flush and sealing times. A few empty test pouches were filled and water displacement/package volumes were tested for

consistency. The pouch sealer was set to pull a vacuum for 6 seconds, flush for 10 seconds and seal for 8.5 seconds.

Hamburgers were removed from the refrigerator, placed into sterile pouches and sealed. Hair nets, gloves, masks, and lab coats were worn by all personnel while handling the beef patties. Gloves were changed between gas mixtures. Packaged samples were stored at 40°F for 4 weeks.

A water displacement test was conducted immediately following packaging to measure gas volume on randomly chosen samples for each gas mixture tested. The water displacement apparatus consisted of a large container with a side spout. A heavy block with a sliding wire attached to it, which has a pull ring at one end and a clip at the other, was placed into the container. The cell was filled to a certain level with water and allowed to equilibrate to room temperature overnight. The sample pouch was clipped to the wire and slowly drawn down into the water. The water was displaced by the pouch and overflowed through the spout into a graduated cylinder for a set period of time, between 3 and 5 minutes. The water volume was recorded, pouch removed, container refilled and repeated for next samples. Water displacement was run for 4 to 12 random samples from each gas mixture and the set period of time was 3 minutes.

Initial microbiological analysis was run on seven random raw hamburger samples, four random cooked hamburger samples, and on one random sample for each gas mixture after packaging. Microbiological analysis was run after 4 weeks storage at refrigerated temperatures on four random samples from each gas mixture. Microanalysis included aerobic and anaerobic plate counts.

Gas analysis was run initially and after 4 weeks storage on two random samples from each gas mixture.

3f. Trial 5 : NATICK MAP EGGS

MAP eggs were studied to evaluate packaging material and gas changes within that material. Cooked scrambled eggs were packed in different modified atmospheres, which were analyzed over time. Changes in gas mixture are dependent on the gas permeability of the packaging material and interactions with the packaged food.

Raw whole eggs were washed by dipping into 100 ppm Clorox solution, agitated, strained and then dipped into clean sterile water. Eggs were removed and dried on sterile paper towels. All utensils that would come into contact with the egg, such as mixing bowls, whip attachment, ladles, spoons, paper towels and parchment paper were presterilized. All surfaces were wiped down with Clorox (R) solution and covered with sterile paper prior to breaking eggs. All personnel wore clean lab coats, hair nets, masks, and gloves with frequent changing. Eggs were individually broken into a small bowl first to prevent possible contamination of the batch. Shells were removed with a sterile spoon. Eggs were then dropped into a large sterile bowl which was kept covered. The eggs were scrambled by beating with the presterilized whip attachment.

One cup of egg was ladled into small loaf pans. These premeasured individual servings of eggs would help limit the amount of handling such as cutting to uniform weight after cooking to reduce contamination. They were then covered with foil and baked in a convection oven at 325°F for 10 minutes. Immediately after baking the eggs were slipped out of the loaf pans onto sheet pans with parchment paper, covered with another sheet of

parchment paper and chilled for 25 minutes in a blast freezer.

Each preportioned scrambled egg was packaged in high barrier material using a Tiromat horizontal form fill sealing (HFFS) machine (Fig. 3). The HFFS rollstock packaging machine accommodates a thermoformable bottom packaging material, which is thermoformed on line using a plug assist, heat and vacuum. The base of the thermoforming plate was modified to impress uniformly spaced indentations to increase contact of gas with packaged food. The formed package is moved down the line where it is filled and after which is coupled with the lid stock material. It next moves into a chamber where it is vacuumed/flushed with the gas mixture and then sealed.

Precautions were taken to assure the quality of the packaging operation. The packaging line was wiped down with the Clorox solution, covered with saran and white paper to reduce dust and contaminates, etc., prior to packaging. Micro plates were run through the packaging line and sealing chamber simulating the normal packaging procedure to check the line for contamination. Packaging trials were run to make control adjustments, such as thermoforming temperature, depth of forming plug, vacuum dwell, flushing time and the sealing temperature. Empty packages were run through the packager and the gas mixtures analyzed for accuracy of delivery.

The high barrier packaging material used was manufactured by Curwood of Wisconsin. The bottom material was a semirigid thermoformable clear packaging film called Curform (R) grade 7748. It is a multilayered composite film of which the outer layer is 17 mil of polyester or PET, a thin polyvinyldichloride or PVDC core layer and 2 mil of polyethylene or PE sealant as the inner layer for a total thickness of 19 mil. It has excellent oxygen barrier properties [less than (1.0 cc/100 sq in)/24 h @ 73°F and 0% RH] and good water barrier properties [less than (0.2 g water/100 sq in)/24 h @ 100°F and 90% RH]. The top material or the lid stock was a flexible nonforming clear anti-fog web called Curlam (R) grade 8057-K composed of 50 gauge polyester on the outside, PVDC adhesive in the middle and 3 mil of anti-fog poly on the inside giving it a thickness of 3.6 mil. This packaging material has excellent oxygen barrier properties [between (0.5-0.8 cc/100 sq in)/24 h @ 73°F and 0% RH]. It also has good water barrier properties [less than (0.3 g water/100 sq in)/24 h @ 100°F and 90% RH]. The packaging materials were tested in house against similar packaging material for oxygen transmission rate and thickness.

The bottom film was thermoformed into a container with the dimensions 5 1/8" x 10" x 1 3/4" into which the preportioned egg was packaged and vacuum/gas flushed with two gas mixtures, one (25% carbon dioxide, 75% nitrogen) and two was air control. The egg portions were transported to the packaging room in a semifrozen state. The eggs were covered at all times. The researchers wore gloves, masks and hats at all times while transporting and packaging eggs. Although the packaging room was not a designated sanitary area, all precautions were taken to minimize possible sources of contamination by following HACCP guidelines. Eggs were cartoned, labeled and stored at 40°F for 6 weeks. Initial gas analysis was run on two samples each for the control air, test mixture and empty gas filled sample. Initial microbiology was run on the raw shelled egg, and on two samples each for the control air and the test mixture. The cooked, packaged egg was also tested for chemical composition.

Samples were withdrawn once a week over the six week period for gas analysis and microbiological analysis. Gas analysis was tracked over time

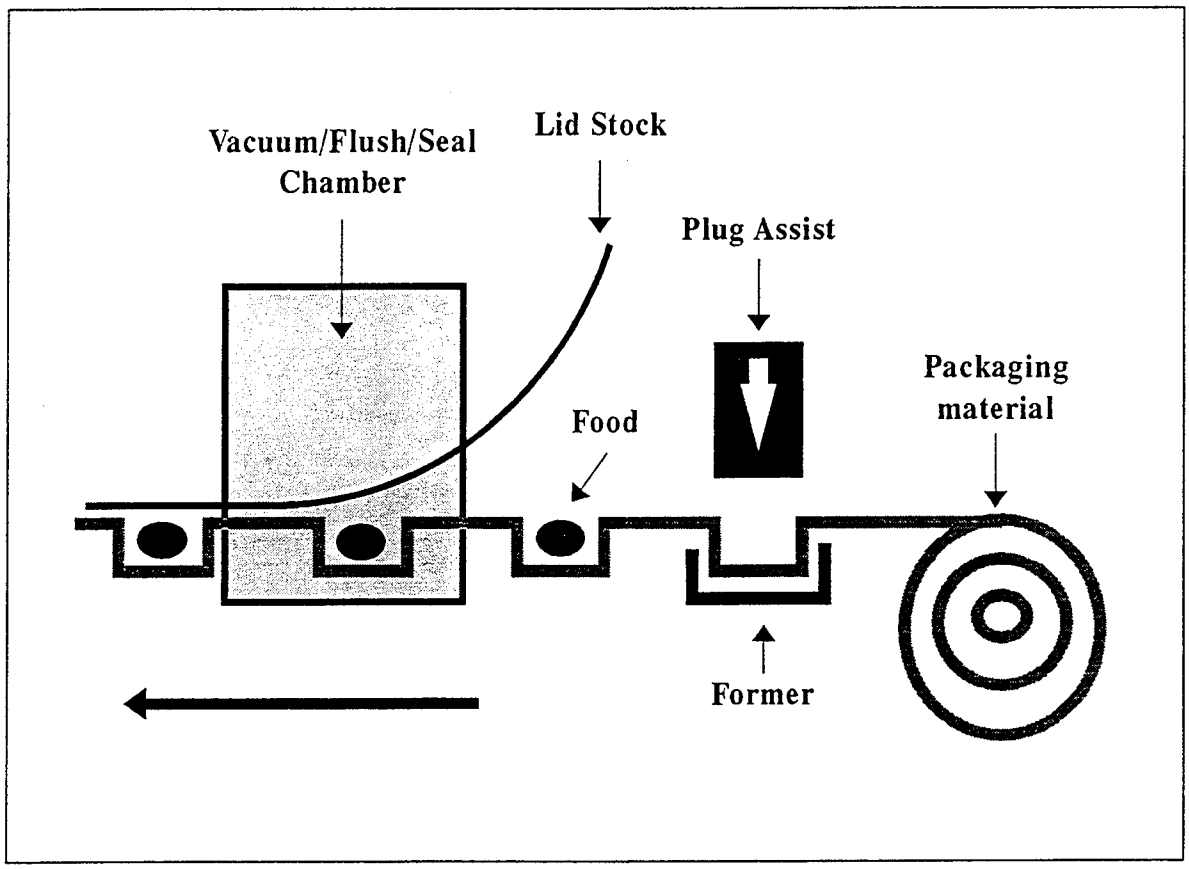

Figure 3. Basic parts of the form/fill/sealing packaging equipment used to package the MAP eggs.

on held samples (gas analysis was run on the same sample at each withdrawal) and on randomly chosen samples at each withdrawal for both control and test gas mixture. Microbiological analysis and pH were conducted on randomly chosen samples, two each for the control air and test mixture at each withdrawal. After 3 weeks storage, random samples of gas filled, control air filled, and a control fresh cooked eggs were tasted for sensory analysis.

3g. Trial 6 : DELUCA'S MAP EGGS (Deluca's, Derby, Connecticut)

Having tested the MAP eggs in-house the next step was to test production at a MAP manufacturer. A local MAP manufacturer was contracted to produce MAP-packed eggs on an experimental basis. This was done because the facilities available at Natick were not set up to produce a microbial-sensitive product such as MAP. There was also a need to establish a process protocol for MAP production of egg. Deluca's (Derby, CT) was contracted because they have a very diverse MAP production line and they are also local to New England.

Deluca's was contracted to provide services which included purchase of ingredients, gas mixtures, labor, recommendations, additional supplies for production of a specified quantity of MAP packed scrambled eggs, and shipment of final product to Natick. The test included packing 32 each multiportion trays of eggs under three test gases and control air giving a total of 128 packages. Also 78 each single-serve trays were to be packed under three test gases and a control air for a total of 312 packages. The agreement also included that a room be designated for the test and to run surface sanitation tests prior to testing. They opted to run the test over the weekend to fulfill the location requirement.

We visited the plant to observe their operation. The ingredients, supplies, gas mixtures, test day and production procedures were also decided. Preliminary cooking tests were run both at Natick and Deluca's to determine the volume of egg, pan size, cooking time and temperature for both a single-serve and the multiserve eggs. It was decided to use frozen pasteurized eggs, which come in 5-gallon drums and are prescrambled. The plant had two convection ovens with settings for hot, hot steam and steam. The packaging equipment was located in the clean room and connected to the chill/freezer room. The multiserve eggs were cooked in a large disposable aluminum pan and gas flushed in microwaveable bag or a 'boil-in-a-bag' with an oxygen transmission rate of (3.3 cc/sq m)/24 h for easy preparation. The single serve eggs were cooked and chilled in a small aluminum pan then transferred and packaged in clear thermoformed containers. The bottom thermoforming material used was a polypropylene with an EVOH barrier with water transmission rate of (0.2 g/100 sq in)/24 h at 95°F/ 90% RH, and an oxygen transmission rate of (0.34 cc/100 sq in)/24 h at 74°F. The lid film was a composite nylon with a barrier layer and polypropylene, a water vapor transmission rate of (0.18 g/100 sq in)/24 h and an oxygen transmission rate of (0.03 cc/100 sq in)/24 h. Plain scrambled eggs with no water or spices added were cooked in a convection oven for 20 minutes at 325°F and chilled for 30 minutes until the temperature reached 35-40°F. The test gases were purchased as premixed gases. The eggs were packaged under Gas 1 (control air), Gas 2 (40% carbon dioxide/ 60% nitrogen), Gas 3 (40% carbon dioxide/ 10% oxygen/ 50% nitrogen), and Gas 4 (25% carbon dioxide/ 75% nitrogen). Due to the high level of activity during normal working hours the tests were run on a

weekend. This schedule would also reduce amount of possible contamination.

The frozen eggs started to defrost in the refrigerator approximately three days prior to the test. The multiserve eggs were processed first. The semifrozen eggs were dumped into a large sanitary bowl and mixed with sanitary utensils. Samples for microbial analysis were taken from each container of raw eggs and stored in the freezer in sterile bags. We test cooked the multiserve eggs to determine the best cooking temperature, time and mode for the convection/steam ovens. Twenty eight oz of egg were ladled into 10.5" x 8" disposable aluminum pans and placed into a shelving unit which rolled directly into the oven. Four trays on each of seven shelves covered lightly with an aluminum lid were cooked at 220°F with full steam, the lids removed and cooked for an additional 5 minutes on dry heat. Four batches were run. The whole shelving unit was rolled into the blast chiller until the eggs reached 40°F or less. Egg samples were taken and frozen just prior to packaging for microbiological analysis. The whole shelving unit was rolled into the adjoining clean room for packaging. Each tray was placed into a bag and vacuum flushed and sealed on a vacuum sealer. Twenty-eight trays of egg for each test gas were MAP packed, boxed, labeled, stored at refrigerated temperature and shipped to Natick. Samples frozen for gas analysis along with the frozen micro samples were also shipped.

The single-serve egg production was postponed for seven days until the following Saturday. Unfrozen pasteurized prescrambled eggs with citric acid added as a preservative were purchased during this time for single serve eggs production. The liquid egg was poured into sterile bowls for evaluation. A sample was taken from each container of raw egg and frozen for microbiological analysis. A volume dispenser was used to dispense 8 oz of egg into a 4.5" X 3.5" disposable aluminum pan. The pans were stacked onto the rolling shelving unit. Fourteen pans on each of seven shelves were covered lightly with foil and cooked at 220°F on combination dry heat/steam for 20 minutes, and for an additional 5 minutes uncovered. Four batches were run. The eggs were rolled into the blast/chiller for approximately 30 minutes. Egg samples were taken and frozen just prior to packaging for microbiological analysis. Eggs were rolled into the clean room for packaging on the form/fill/sealing unit similar to one used for the in-house eggs described above. The egg blocks were transferred from aluminum pan to the thermoformed container and sealed under control air and the same four test gas mixtures used for the multiserve eggs.

Alternating gas mixtures with and without oxygen allowed for indication of complete flushing of gas through packaging equipment by oxygen analysis between each test gas. Equipment settings for vacuum time were 5 seconds at 92 psi to 2 millibars and gas flush time was 2.5 seconds. Ninety pans of egg for each test gas were MAP packed, boxed, labeled, stored at refrigerated temperature and shipped to Natick. Samples frozen for gas analysis along with the frozen micro samples were shipped also.

The initial analyses were not run until approximately one week after the day of production. Microbiological analyses were run on frozen raw, cooked prior to packaging and initial MAP packed samples from each gas mixture for single-serve eggs and again after 2 months. Gas analyses were run on initial samples and again after 4 weeks from each gas mixture for the single-serve eggs.

4. RESULTS AND DISCUSSION

4a. Trial 1 : NATICK MAP CHICKEN

The most significant observation made during this study was that there was very little change in the gas mixtures over time. The data and a graph showing this stability can be found in Figs. A-1a...1e and Table B-1. Statistically there were no significant differences in the composition of Gas 2 (40% carbon dioxide/ 5% oxygen/ 55% nitrogen) and Gas 4 (60% carbon dioxide/ 40% nitrogen) over the 5-week period. There were some significant differences found between earlier weeks and later weeks for Gas 3 (40% carbon dioxide/ 60% nitrogen) but there were no trends. There were significant differences found over time for both Gas 1 (40% carbon dioxide/ 2% oxygen/ 58% nitrogen) and the Air Control. An increasing trend was seen for carbon dioxide (from 24% initially to 26% after 5 weeks for Gas 1 and from 0.93 initially to 8.6 after 5 weeks for air control) and a decreasing trend was seen for oxygen from 2.97 initially to 0.13 after 5 weeks for Gas 1 and from 17.67 initially to 1.2 after 5 weeks for air control. There was also an increasing trend seen for nitrogen in the air control.

Although there were statistically significant differences seen for some of these groups, the gas composition is considered to be quite static except for the control. This result shows that the high barrier jars with their modified lids were airtight. The lower carbon dioxide levels may be due to initial carbon dioxide absorption by the chicken. The low levels also show that after initial absorption cooked meat such as chicken does not interact with the gaseous environment within a high barrier jar, leaving the gas mixture virtually unchanged during cold storage.

High levels of contamination were found after 1 week storage. Levels ranging from 2.5 to >18 million colony forming units (CFU)/g of sample for aerobic plate counts were found and between 6,500 to 201,000 million CFU/g of sample and TNTC (too numerous to count) for anaerobic growth which were considered to be microbiologically unacceptable, with the exception of Gas 4 (60% carbon dioxide/ 40% nitrogen) which had relatively low levels of contamination only 135 aerobic and 1,700 anaerobic plate counts. Microbiological analysis was run after 2 weeks storage but was discontinued after that due to very high counts found in all samples. Escherichia coli and Salmonella were both found to be negative during the microbiological testing period. Because there are no existing standards for MAP, microbial limits are based on the initial microbial count, which should be <10 million CFU/g of sample. If there is any consistent microbial growth found, then the product is considered to have failed.

High levels of contamination may be due to difficulty encountered when injecting gas mixtures. Gas filling was found to be lengthy and tedious. The gas filling operation took approximately 5 minutes per jar and was conducted in a lab with no refrigeration. Although the chicken were sealed in the glass jars and the needles were replaced periodically for safety reasons, this may have presented an opportunity for contamination. These conditions may have contributed to the contamination problem. The gas filling procedure was found to be quite consistent, although it did not deliver the intended proportion of gases and is also very impractical due to time limitations. The airtight jars were found to be very useful in showing interactions between the food and its gas atmosphere. Since it was not certain where the contamination came from and there was no HACCP

in place, it can be assumed that much stricter controls are needed for a MAP product.

4b. Trial 2 : TRIO'S MAP CHICKEN (Trio's, Chelsea, MA)

Similar to the in-house chicken study described above the gas mixtures remained quite stable during the testing period (Figs. A-2a...2c and Table B-2a). The gas mixtures were however found to be between 50-60% lower for carbon dioxide, 50% lower for oxygen and 33-40% higher for nitrogen than what was injected at packaging. The first gas analysis was not run on these samples until two weeks after packaging due to circumstances beyond our control and there may have been a change in the gas mixture during that time. It is highly unlikely that these drastic changes were due to gas absorption, which can cause a decrease in gas volume and gas composition shortly after packaging; however, the changes may be due to inaccurate gas injection. The use of a high barrier forming material prevents any gas exchange between the outside atmosphere and the gas mixture within the package.

There were high levels of aerobic and anaerobic growth found after only 2 weeks of refrigerated storage. No growth was found for both Escherichia coli and Salmonella (Table B-2b). It was thought to be the excessive handling of the product after cooking and prior to packaging that caused the high contamination. These chilled products are highly sensitive to contamination when dealing with nonsterile conditions and there is a great need to establish some Hazard Analysis Critical Control Point program elements in the processing procedure to minimize the microbiological problems encountered so far.

4c. Trial 3 : EVALUATION OF TIME TEMPERATURE INDICATORS (TTIs) AND COMMERCIAL MAP ITEMS

The sensory data were analyzed statistically for significant differences. For both chicken and lasagna there were no significant differences over the sensory testing period for odor, flavor, texture and overall. There were, however, significant differences found between the Week 1 and Week 2 appearance ratings for chicken and lasagna. The individual appearance ratings for chicken ranged between 7.0 and 5.5 during the study. The individual appearance ratings for lasagna ranged between 7.0 and 5.6 during the study. Averages of six ratings were used for statistical analysis and are tabulated in Table B-3d. Sensory ratings using the line scale for chicken showed a large variation in individual ratings but when the ratings were averaged showed no extremes. There was a very slight trend towards the unacceptable range over time.

The microbial data averages were converted to their log values. An average of three measurements were used for the statistical analysis. In addition, the microbiology lab reports an average of two repetitions. There were no statistical differences between the time periods tested for microbial growth in the chicken. Although there was one 100°F sample out of three which had a high aerobic plate count of 85 million CFU/g after 2 weeks storage, the average plate count ranged from 0 to 30 for the others. This may have been the 1 in a million which slipped by the HACCP procedures or quality control at the chicken processing plant, to which improvement may be needed.

The lasagna microbial data showed significant differences between

control/1 week samples and the 1 week abuse 100°F/2 week samples. Microbial growth was noted soon after the abuse period and randomly increased to unacceptable levels following that period. The data used for statistical analysis can be found in Table B-3e. The microbial data were found to be quite random and we were not able to draw any conclusions. Yeast and mold counts remained at <10 CFU/g of sample throughout the study. The pH ranged between 5.75 to 6.7 for the chicken and ranged between 5.09 to 5.67 for the lasagna, Table B-3f.

Averages of six TTI scans for each label were collected from 4 to 7 labels in each group. The averaged reflectance data for chicken and lasagna with no abuse, abuse at 70 and 100°F can be found in Tables B-3g...3i. The time temperature indicator data show a consistent decrease in the reflectance reading for all label types over time for the no abuse groups for chicken and lasagna. All TTI averaged data were significantly different from one another and when graphed show a relatively straight declining line. (The slopes for the no abuse lasagna data were -13.98, -20.99, and -25 for label #60, #67, and #76, respectively.) The slope data can be seen in Table B-3j.

The different labels are formulated to react to time and temperature at different kinetics or rates of speeds. The label that is least sensitive to time and temperature is #60. This can be seen by its larger slope and the initial reflectance ranges of 90/100 that decrease to around 50 after 3 weeks at 35°F. The most sensitive label is #67 if looking at the average reflectance or label #76 if considering the slope. Label #67 starts at a reflective range of 60/70 and decreases to 50 after only 1 week and reaches the 0 range after 3 weeks. On the other hand label #76 starts at 70/80 and decreases to the 50 range after 2 weeks and reaches the 0 range after 3 weeks. It is apparent that there is a difference in the rates at which the label reflectance approaches 0. This is shown in graphical form for lasagna in Figs. A-3a...3c. The difference in the starting ranges can be attributed to its sensitivity to the drastic change from deep freeze to room temperature during the time it took to attach labels to samples and the initial reflectance reading. Similar, steadily decreasing reflectance readings are found over time for the chicken and lasagna stored 70°F abuse group; however, there was no significant difference found between the 1 week samples and the samples that were abused that very same day for 4 hours at 70°F with the exception of label #76 for lasagna. The stored 100°F abused group reflectance reading steadily decreases and all are significantly different over time including 1 week samples and the samples that were abused for 4 hours at 100°F. It is desirable to see a difference between these two variables, which in effect shows a sufficient change in the reflectance to warn that temperature abuse has occurred. The slopes for the 70°F abuse lasagna data were -13.56, -14.8, and -17.8 for label #60, #67, #76, respectively. The slopes for the 100°F abuse lasagna data were -15.55, -15.75, and -21.08 for label #60, #67, and #76 respectively. The slopes indicate that the most sensitive label to abuse is label #76. The correlation coefficients for the slopes were all very high and are listed with the slopes in Table B-3k.

The drastic differences in microbial growth between the chicken and the lasagna make it difficult to compare microbial growth with reflectance data. After 2 weeks storage the lasagna showed consistent indications of microbial growth while the chicken showed high growth in one out of 6 plates. We must consider the worst and say that products with potential

for significant bacterial growth such as the lasagna may be typical of a commercial MAP item.

Label #60 is appropriate for a nonabuse situation or as an expiration indicator after 4 weeks for a MAP item such as the chicken, assuming that one-time growth was a fluke. None of the labels with the exception of label #76, for lasagna only, was sensitive enough to indicate an abuse for 4 hours at 70°F. Label #60 indicated slightly for the 100°F abuse, label #67 showed a range of reflectance after the 100°F abuse and label #76 showed the most definite change after abuse at 100°F. It is quite noticeable that there is a difference between the chicken and lasagna label #76 reflectance in the abuse groups. This may be due to the different packaging types. The lasagna was packaged in a high-barrier semi-rigid thermoformed container within a cardboard carton and the chicken was packaged in flexible, high-barrier vacuum sealed packaging. Abuse for 4 hours may not have been sufficient to parallel real life situations.

4d. Trial 4 : NATICK MAP POUCH HAMBURGERS

Although a preliminary test for gas volume showed a consistent gas fill, there were, however, large variations in the gas fill for samples prepared for this study (Fig. A-4a and Table B-4a). The water displacement data show a large difference in gas volume in pouches within groups. The greatest difference found within a group was 606 cc. There were very high differences found in four of the eight groups. These differences were due to faulty pouch sealing equipment. The pouch sealing equipment was delivering highly variable gas volumes. Since the volume differences were unexpected, the water displacement test procedures were modified slightly in an attempt to get more consistent results. Increasing the waiting period increases the accuracy of the water displacement test. The waiting period was increased from 3 minutes to 5 minutes and then again to 10 minutes because it was uncertain as to whether it was the gas volume or the water displacement test method that was inconsistent.

Gas analysis results for Week 0 and Week 4 samples were compared. The data show no drastic changes in gas composition after four weeks of refrigerated storage. Statistically, however, the percent oxygen in gas mixtures 3, 4 and 6 were found to be significantly different, with a decreasing trend from Week 0 to Week 4 (Figs. A-4b...4c and Table B-4b). Gas analysis also showed that gas compositions were different than what was injected but the difference was relatively small and like samples were consistent. It appears that the varying gas volumes between samples had no effect on the gas compositions initially or over time.

Microbiological analysis run on the raw samples varied greatly between containers but all were within acceptable ranges. Aerobic plate counts for raw samples ranged between 3,950 - 111,000 CFU/g of sample. Cooked samples all contained aerobic and anaerobic plate counts of <10 CFU/g of sample (Table B-4c). The Week 0 gas-packed samples contained aerobic plate counts of $\leq$10 CFU/g of samples and they were all found to be acceptable (Table B-4c). There was mold growth as well as microbial growth found in the control air sample after four weeks storage at refrigerated temperatures. There was no anaerobic plate growth in samples packed under Gas 1, 3, and 6 after four weeks storage. There was very little aerobic plate growth in Gas 3 samples. There were significant

anaerobic plate growth and <10 CFU/g found in samples packed under Gas 2, 4 and 5. There were also plates with significant aerobic plate growth and <10 CFU/g found in samples packed under Gas 4 and 5. It is difficult to draw any conclusions from these data.

4e. Trial 5 : NATICK MAP EGGS

Initial gas analysis run on the in-house MAP eggs shows that the gas composition delivered by the packaging equipment was slightly off but very consistent from sample to sample. The test gas mixture injected was 25% carbon dioxide/ 75% nitrogen and the average resulting gas compositions was 19.35% carbon dioxide/ 0.6% oxygen/ 80.05% nitrogen. The control air samples tested similar to atmospheric air. Gas analysis was run weekly on random and held samples for both the control and the test gas samples throughout the study. The data show interaction between gas atmosphere and the food or exchanges with atmospheric air (Figs. A-5a...5d and Table B-5a). The data show very weak trends towards decreasing carbon dioxide and weak trends towards increasing nitrogen for both held and random test gas samples and for the control air random samples. There were also weak trends found in the control air random for decreasing oxygen and the test gas held samples showed slight increasing trend for oxygen. The gas mixtures are considered to be very static because the initial gas analysis was run immediately after packaging and was found to be close to what was injected. Also there were very slight changes in the gas composition during the test period, although there were significant differences found statistically.

The high barrier, semirigid thermoforming packaging material was found to be suitable for this study. When the Curform (R) film was analyzed for oxygen transmission rate it ranged between (3.78-3.89 cc/sq m)/24 h for the top film and (2.51 cc/sq m)/24 h for the thermoforming bottom film (Table B-5b). A micrometer was used to measure the average thicknesses, which was found to be between 3.2 and 3.6 mil for the lid film and between 20 and 20.7 mil for the thermoformed bottom. The spec for these films quoted 3.6 mil for the lid and 19 mil for the bottom.

The chemical composition of an egg sample was analyzed and included protein, fat, moisture, and ash contents along with pH and Aw, shown in Table B-5c. The main interest was for moisture and fat percentages. A high percentage in moisture level may cause a syneresis or separation of the water over time. A high percentage in the fat content may cause oxidation when packed in gas mixtures with oxygen.

The control air samples were analyzed for aerobic microbes and the test gas samples were analyzed for anaerobic microbes during the test period (Table B-5c). There was no anaerobic growth beyond 10 CFU/g of sample over the 6-week period for the test gas samples. There was, however, aerobic growth seen after 3-weeks storage for the control air samples with the highest levels seen after 4 weeks and then decreasing slightly after that. All samples were analyzed for Salmonella, Escherichia coli, coliforms, and Staphylococcus aureus weekly and found to be negative during the 6-week study. All test gas samples were within the microbial acceptable range. The control air samples were microbially unacceptable after 3 weeks and mold growth was observed after 5-weeks storage. Microbial growth found in the control air sample at 4 weeks was significantly different from all other weeks. It is impossible to compare the two groups tested in this study for microbial growth due to the fact

that for the control air sample the aerobic microbes were tested and for the gas test sample the anaerobic microbes were tested. The pH was measured when microbial analysis was run and is tabulated in Table B-5c. The pH ranges seem to be consistently higher for the control air samples.

Sensory testing showed that the stored samples rated lower than the fresh control and the gas test samples rated very slightly less than the control air samples. There was a noticeable green tinge on the bottom of egg samples from both test groups. The odor was low and the flavor was bland with a hard-boiled egg flavor. The texture was moist but very slightly tough.

4f. Trial 6 : DELUCA'S MAP EGGS (Deluca's, Derby, CT)

The multiserve eggs were very pale yellow or yellow with a very slightly green color. The single-serve eggs were a normal color yellow. It was thought that the difference was due to the type of egg used. Eggs without citric acid were used for the multiserve eggs while eggs with citric acid were used for the single-serve eggs.

Initial gas analysis on the multiserve eggs showed that the gas compositions were extremely erroneous. This finding could be due to incorrect use of or faulty gas filling equipment. There may have been severe absorption of carbon dioxide by the egg during the 1-week period before shipment, leaving very little gas behind. When the multiserve packaging bags were tested for oxygen transmission rate it was found to be (3.172-3.442 cc/sq m)/24 h. The oxygen transmission rate data for the packaging films used in this study are given in Table B-6a. Normal gas composition ranges were found for the control air samples. Within the test gas samples there were very low carbon dioxide levels, and high levels of oxygen and nitrogen. For all samples, gas compositions were very similar to the control air. The gas analysis for the multiserve test gas eggs was found to be unacceptable, so no further testing was conducted on the multiserve group. The product was already considered to be severely compromised.

Initial gas analysis on the single serve eggs was also found to be extremely different from what was injected (Table B-6b). The packaging material used for the single serve eggs possessed high moisture and gas barrier properties. The lid material had a oxygen transmission rate of (2.434-3.122 cc/sq m)/24 h. The carbon dioxide levels were somewhat higher then the multi-serve samples but were still very significantly lower than what was injected. The oxygen levels were much higher relative to what was injected. The nitrogen levels were also much higher than what was injected. When Week 1 data were compared to the Week 4 data statistically, there were significant differences found in the oxygen control in air and Gas 3, and also for the nitrogen Gas 2. The gas analysis data can be found in Figs. A-6a, 6b and Table B-6b.

There were aerobic counts between 1600 and 9900 CFU/g of sample, negative Escherichia coli and <10 CFU/g of sample for yeast and mold for all samples of raw egg used in the multiserve production. There were lower aerobic counts between <10 and 555 CFU/g of sample, negative Escherichia coli and <10 CFU/g of sample for yeast, mold and anaerobic microbes for all samples of raw egg used in the single serve production. Initial or Week 1 storage microbiological analysis for the multiserve samples showed no growth of Escherichia coli and <10 CFU/g of sample for all aerobic, anaerobic, yeast and molds plates. Initial or Week 1 storage

microbiological analysis for the single serve samples showed negative results for yeast, mold and E. coli, and <10 counts for all aerobic and anaerobic plates. The Aw for the initial cooked MAP packed samples was between 0.989 and 0.996 for the multiserve group and between 0.984 and 0.991 for the single-serve group. The TBA, moisture, protein, fat and ash data for the single-serve eggs are reported in Appendix B. The pH for the initial cooked MAP-packed samples was between 7.88 and 8.25 for the multiserve group and between 6.84 and 7.05 for the single-serve group.

Deluca's plant was in full production on both test days throughout the entire test, which made it very difficult to conduct the test. It was specified in the contract that there be a designated location that was satisfied by opting to run the test on the weekend. The first test day the eggs were still frozen, which made it difficult to ladle into pans. Removal of lids to return them for an additional 5 minutes of dry heat cook was quite awkward and labor intensive. The eggs were left uncovered while in the blast chiller. The multiserve eggs were a very pale yellow color and stuck to the aluminum pan while the single serve eggs were a yellow color and did not stick to the pan. This could be due to different cooking methods or different egg type.

5. CONCLUSIONS AND RECOMMENDATIONS

MAP gas mixtures with high barrier packages can control the growth of spoilage microbes but they cannot ensure microbiological safety. Intermediate moisture foods may, and high moisture foods must also be kept refrigerated to prevent microbial growth. The growth of harmful anaerobic organisms such as Clostridium botulinum can also occur if there is no oxygen present. Low temperature organisms such as Listeria monocytogenes and Yersinia enterocolitia can cultivate during extended storage at low temperatures.

The hurdle concept, which is a method of adjusting critical factors, can present hurdles for and reduce the chances of microbial growth. Water activity, pH, gas concentrations, initial microbial load, package permeability and storage temperature are some of the critical factors that can be altered. The more hurdles applied the more difficult it is for microbes to overcome these hurdles and grow [33].

There are other key factors that contribute to maintain microbial safety and quality of MAP and all food products. These factors include the use of high quality ingredients, strict sanitation practices, appropriate packaging system and equipment, maintenance of adequate temperature control and appropriate gas mixture. If there are any microbial spores present and there is an opportunity for growth, the quality will be compromised, may cause a health hazard and the shelf life will be shortened. It is important to start with ingredients that have been properly handled with no contamination because MAP products are commonly only minimally processed. Spores are ordinarily killed during retorting or other high-heat preservation techniques.

The lack of refrigeration or increase in temperature during the life of the product could result in microbial growth, which can be inhibited by carbon dioxide and low temperatures. There have been no significant outbreaks due to the use of MAP but this does not mean that infections have not occurred.

The production of MAP packaged foods requires special processing procedures and equipment. It is desirable to have pressurized ultra clean

filling and packaging rooms with air lock entrances and exits for employee gowning, etc. Packaging materials should be UV sanitized and there should also be clean rooms for individual quick-frozen produce and other ingredients [34].

Public safety issues have initiated significant regulatory interest related to Controlled Atmosphere Packaging (CAP)/MAP chilled food technology. At the present time there are few federal regulations that require MAP producers to do anything differently than any other food producer. Meanwhile there is great consumer demand for convenient, minimally processed chilled foods resulting in large growth within the industry under minimum of outside regulatory action. It is the responsibility of the MAP company to establish HACCP principles within its production based on assessing hazards from the growing of raw ingredients to the consumption of the finished product. Critical control points must be determined for each hazard by establishing limits and procedures for monitoring and what corrective action should be taken when a deviation is identified. Each company must tailor and implement a HACCP program based on the different characteristics of its production technologies and distribution. The incentive to establish well-defined and workable HACCP guidelines is marketing success of the product, the degree of risk the producer is willing to undertake and the quality of the product [17].

Storage and distribution logistics are important issues for the military. An efficient and fast distribution system is also an important factor for success in the commercial market. Logistically, many military food rations are purchased and transported to storage units around the world. These storage units must be refrigerated for MAP items. If a MAP food is to be a field ration, it must have refrigerated transportation and be temporarily stored in refrigerated boxes in the field. This type of ration cannot then be distributed amongst the soldiers to carry in their pack and consume as needed because of refrigerating limits.

Based on the studies in this report, unless we can ensure the safety of the current MAP for extended storage time and confirm the safety by using a reliable, rapid microbial test, MAP products don't seem to be ideal for military field scenarios.

Currently the use of MAP within the military has been limited to shelf stable intermediate-moisture products and fresh produce. There is a growing use of oxygen scavengers for baked items in the Meal Ready-to-Eat (MRE), such as the shelf-stable bread. Oxygen scavengers are also used in experimental items including several baked items with meat fillings. The Navy is successful in transporting several kinds of fruit and vegetables overseas by ship within CAP refrigerated vans [35]. Transporting fruits and vegetables overseas under CAP is less costly because water transport is less expensive than the alternative air transport and there is a significant reduction in perished product. CAP also serves as an efficient fumigant and may prove a sufficient alternative to the quarantine method of methyl bromide, which is scheduled to be banned.

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APPENDICES

A. Figures — Trials 1 to 6

B. Tables — Trials 1 to 6

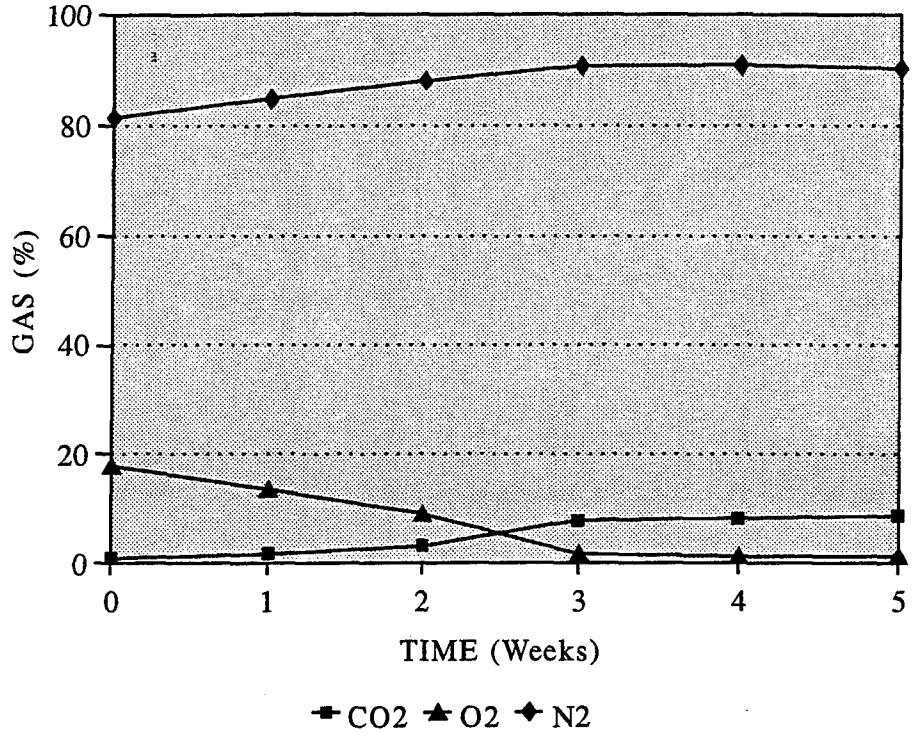

Figure A-1a. Natick MAP chicken: Air (2% CO2; 18% O2; 80% N2) vs. Time

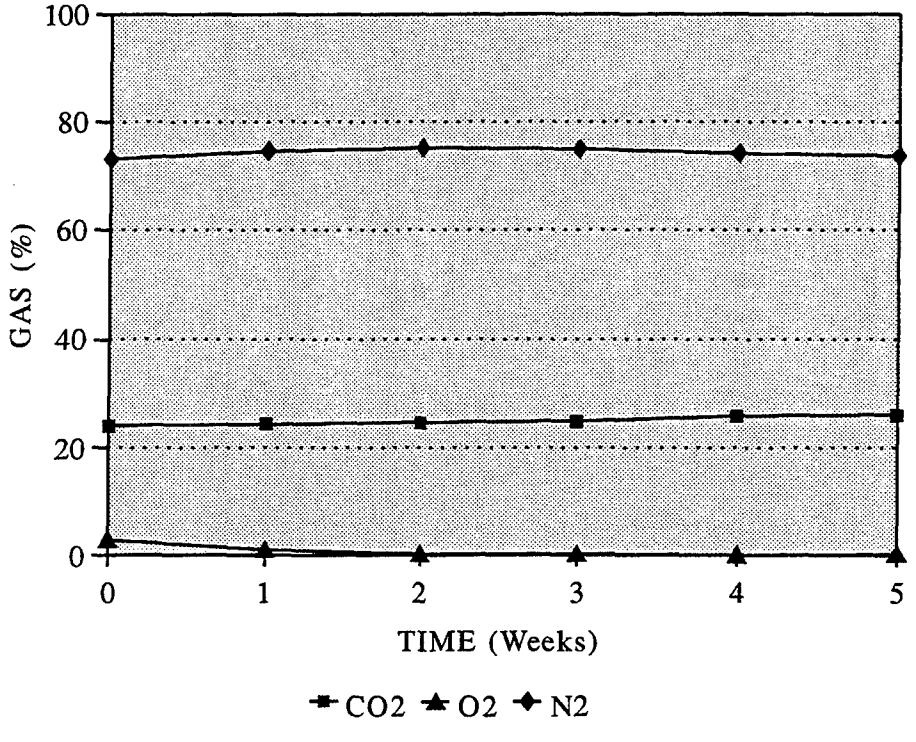

Figure A-1b. Natick MAP chicken: Gas 1 (40% CO2; 2% O2; 58% N2) vs. Time

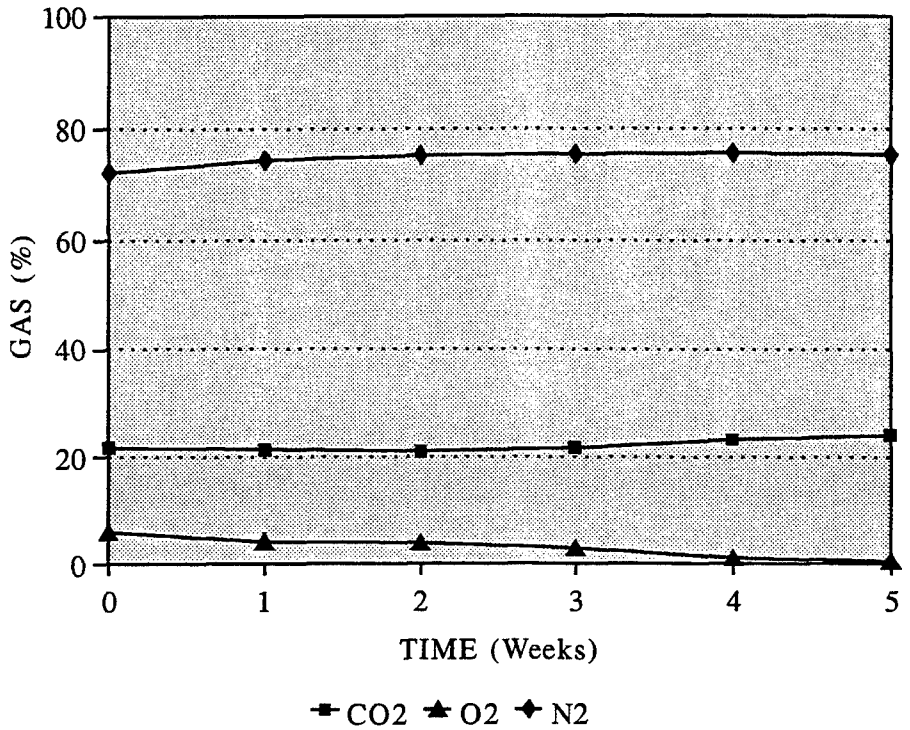

Figure A-1c. Natick MAP chicken: Gas 2 (40% CO2; 5% O2; 55% N2) vs. Time

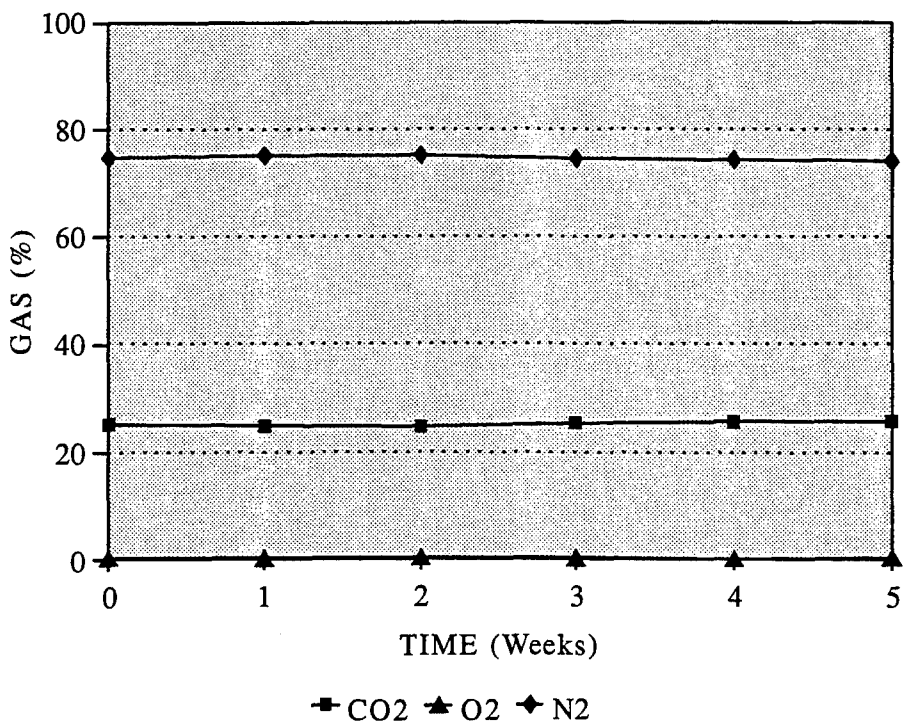

Figure A-1d. Natick MAP chicken: Gas 3 (40% CO2; 60% N2) vs. Time

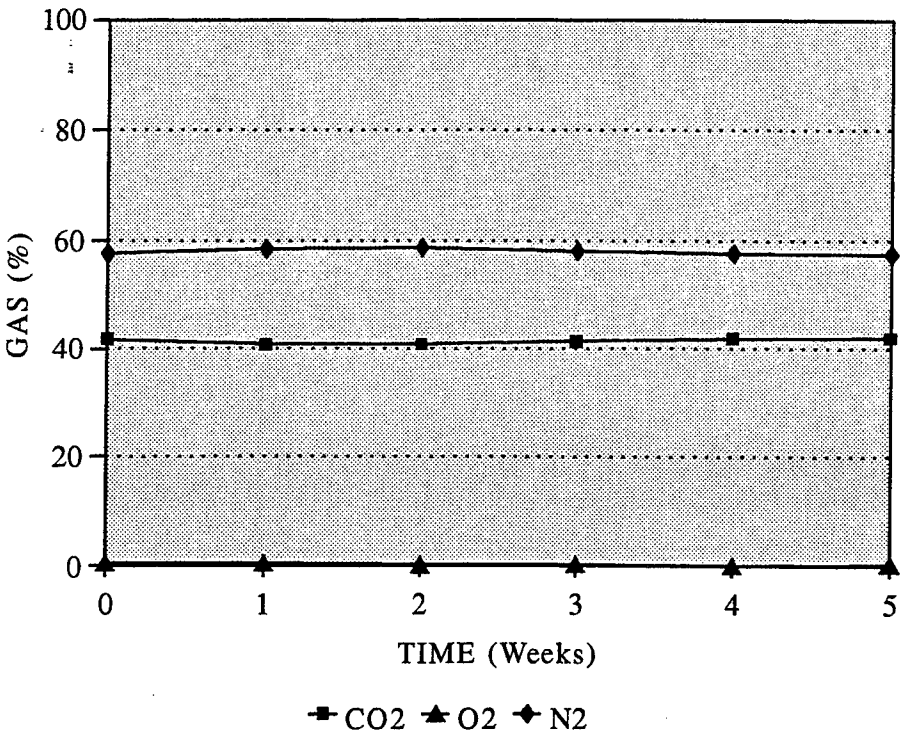

Figure A-1e. Natick MAP chicken: Gas 4 (60% CO2; 40% N2) vs. Time

Figure A-1a...1e show the gas composition over time for the Natick MAP chicken packed in control air, Gas mixture 1, 2, 3 and 4 respectively.

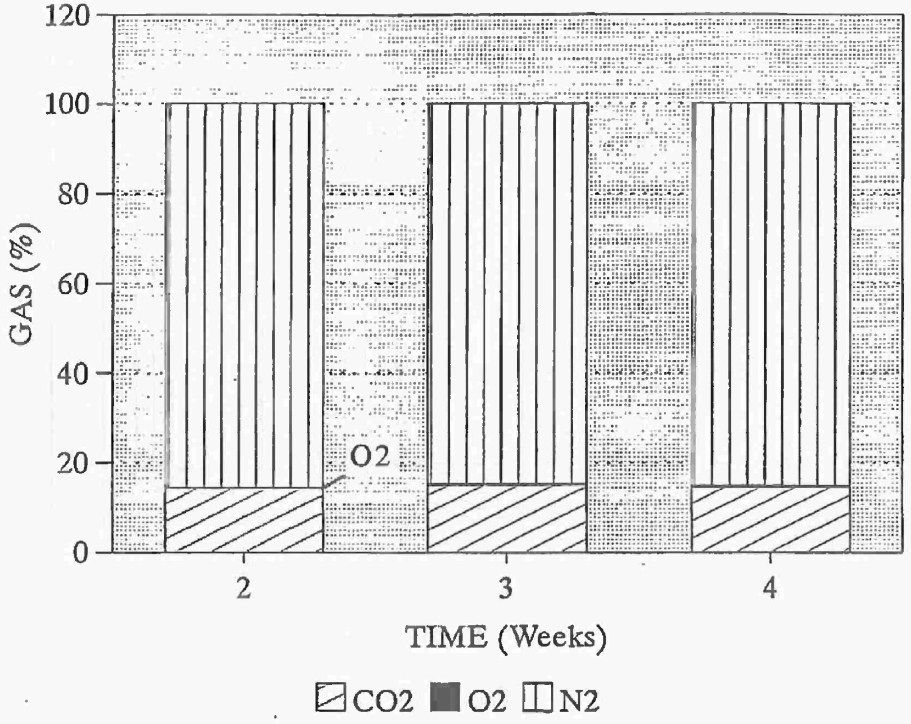

Figure A-2a. Trio's MAP chicken: Gas 1 (40% CO2; 0.5% O2; 60% N2) vs. Time

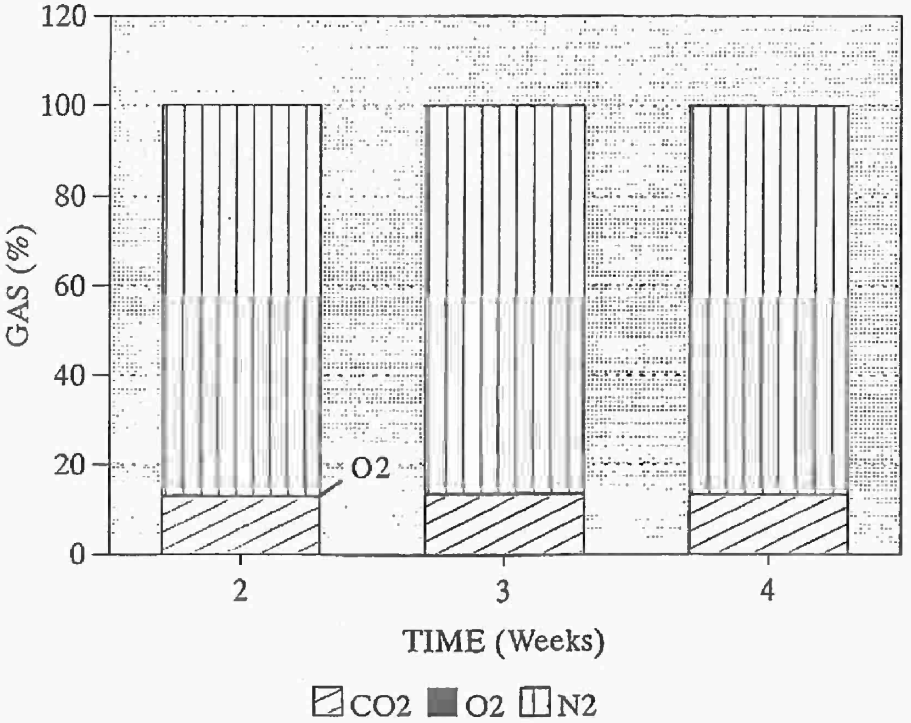

Figure A-2b. Trio's MAP chicken: Gas 2 (40% CO2; 2.5% O2; 60% N2) vs. Time

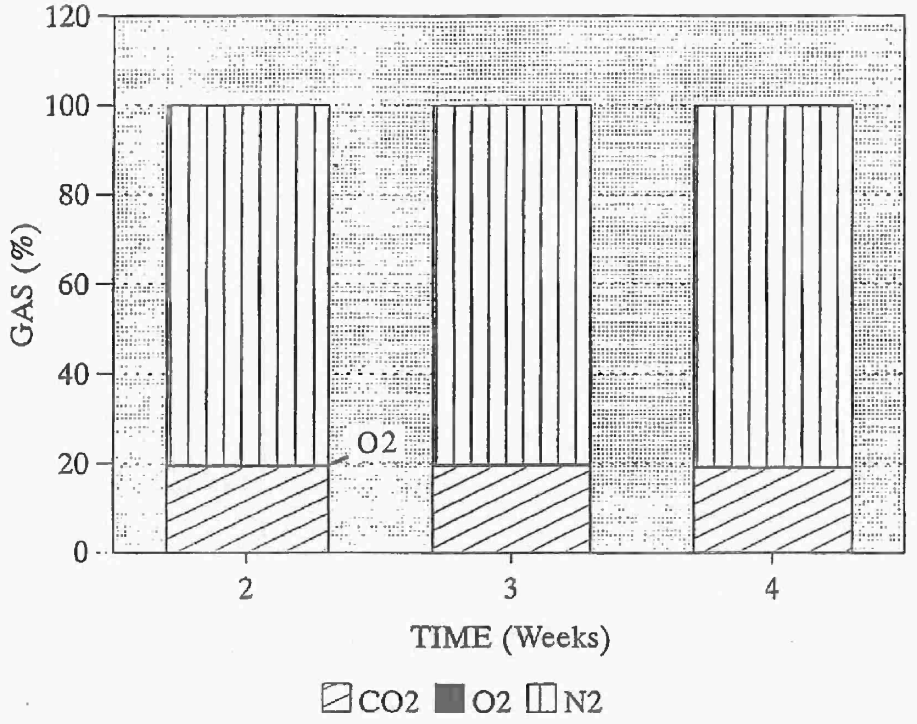

Figure A-2c. Trio's MAP chicken: Gas 3 (40% CO2; 0.35% O2; 60% N2) vs. Time

Figure A-2a, 2b, and 2c show the gas composition during the testing period of the MAP chicken packed at Trio's in Gas mixture 1, 2 and 3 respectively.

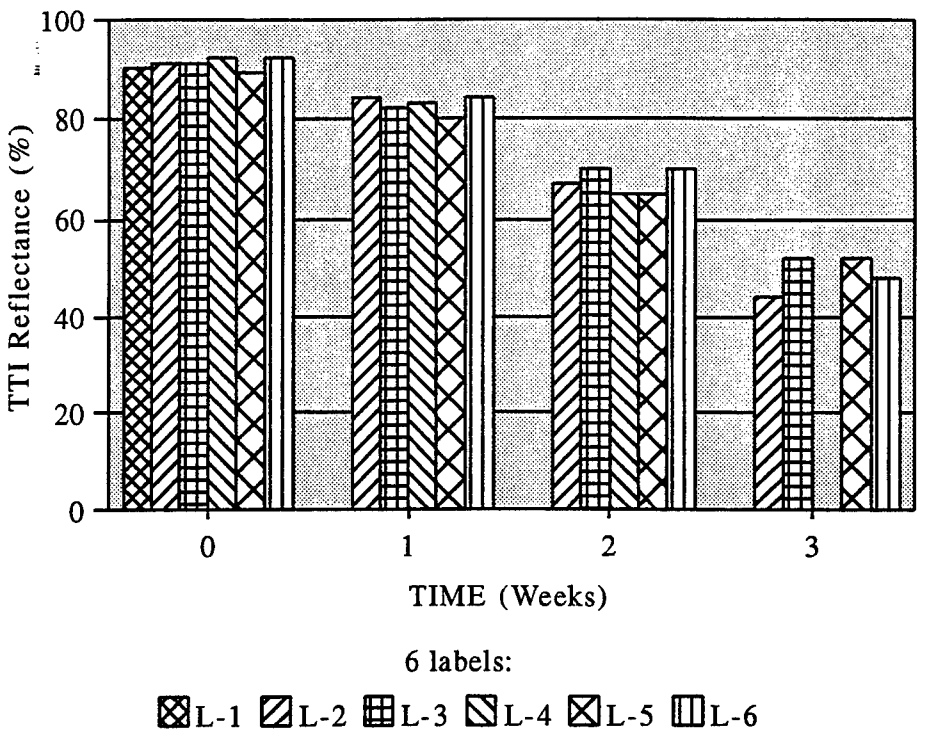

Figure A-3a. Control MAP lasagna TTI label #60 reflectance data

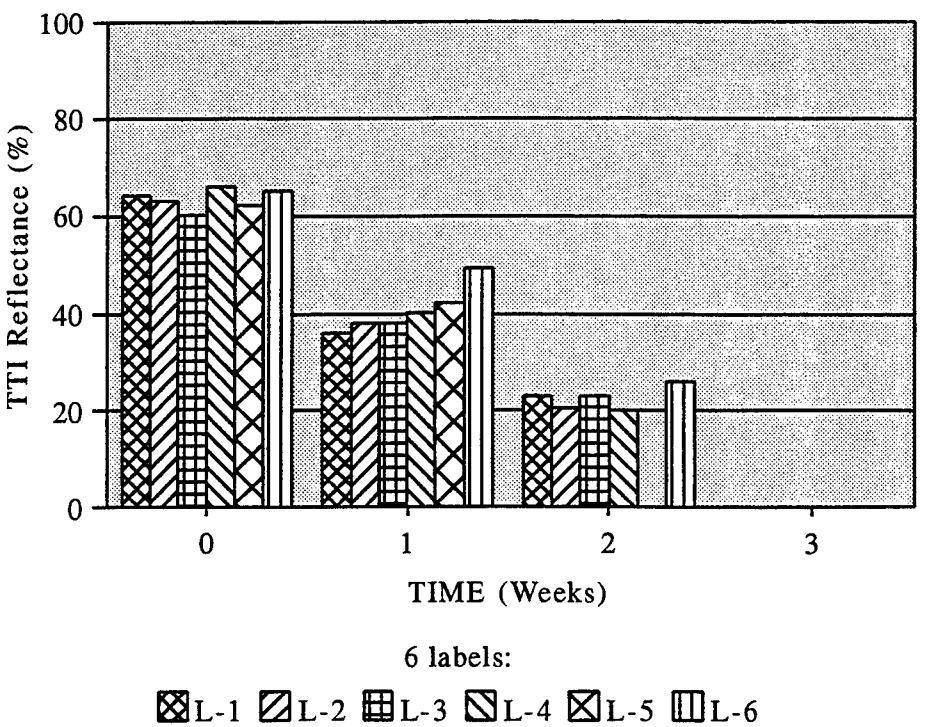

Figure A-3b. Control MAP Lasagna TTI label #67 reflectance data

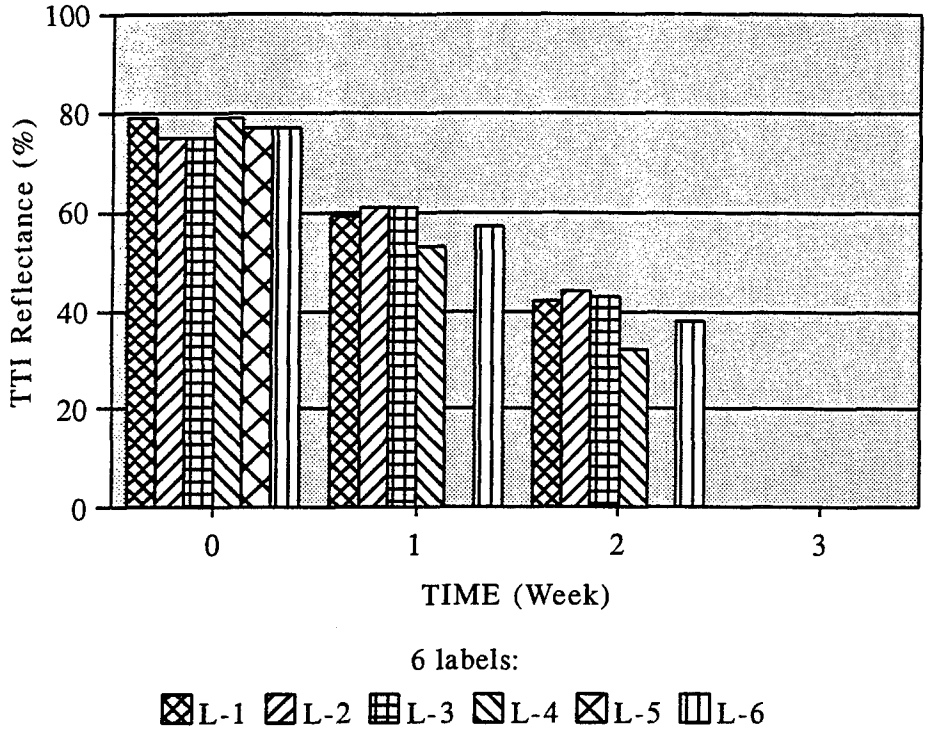

Figure A-3c. Control MAP lasagna TTI label #76 reflectance data

Figure A-3a, 3b, and 3c are bar graphs showing the change/decrease in reflectance of the Time Temperature Indicator labels #60, #67, and #76 respectively which were on the lasagna control samples. Each patterned bar represents one label.

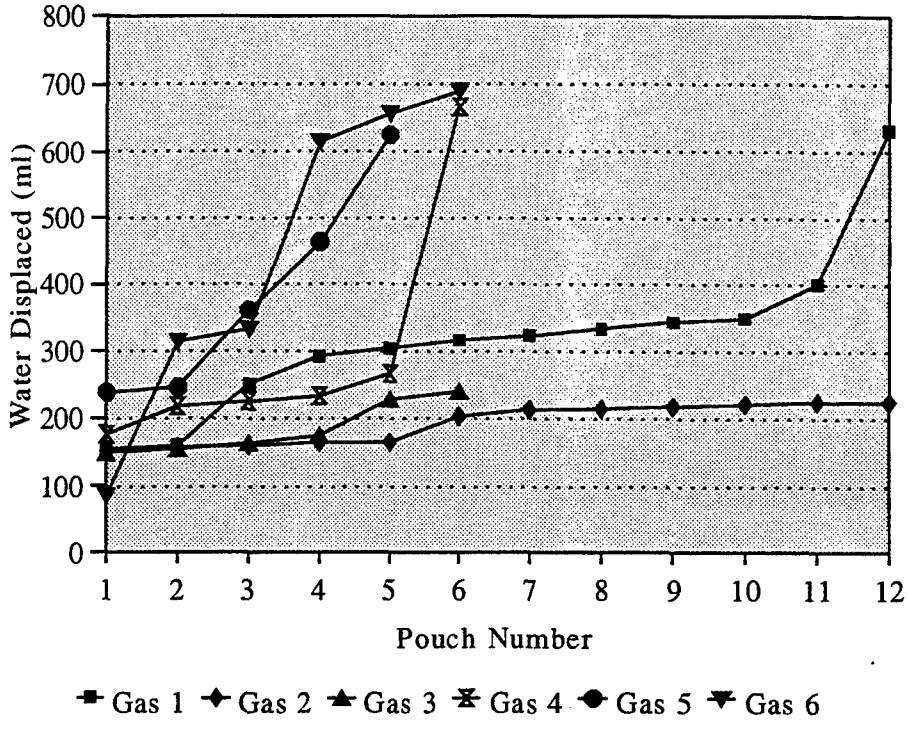

Figure A-4a. Natick MAP hamburgers water displacement data

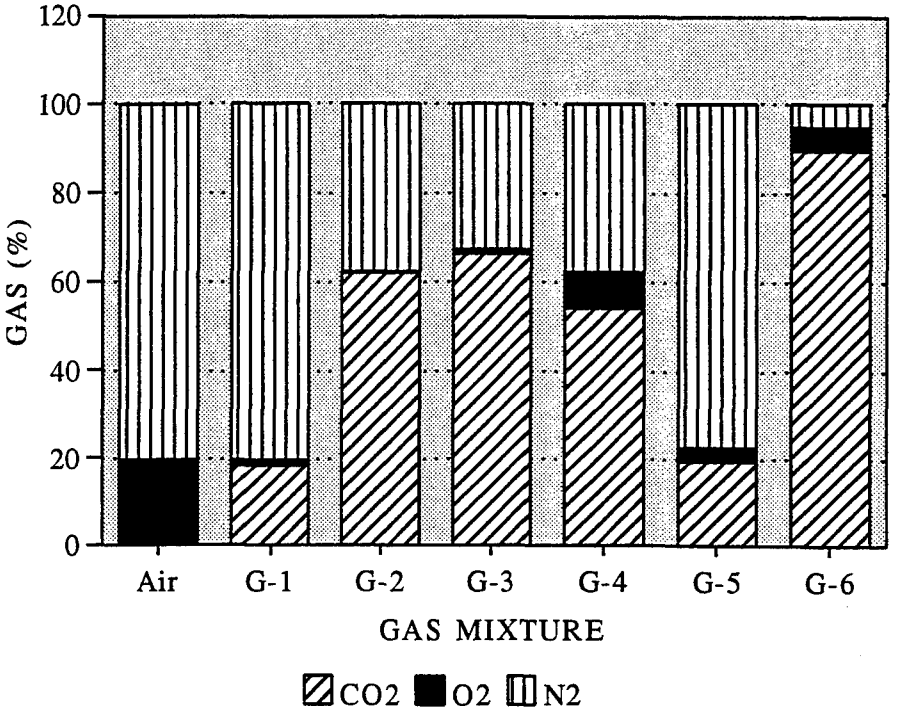

Figure A-4b. Natick MAP hamburgers week 0 gas composition

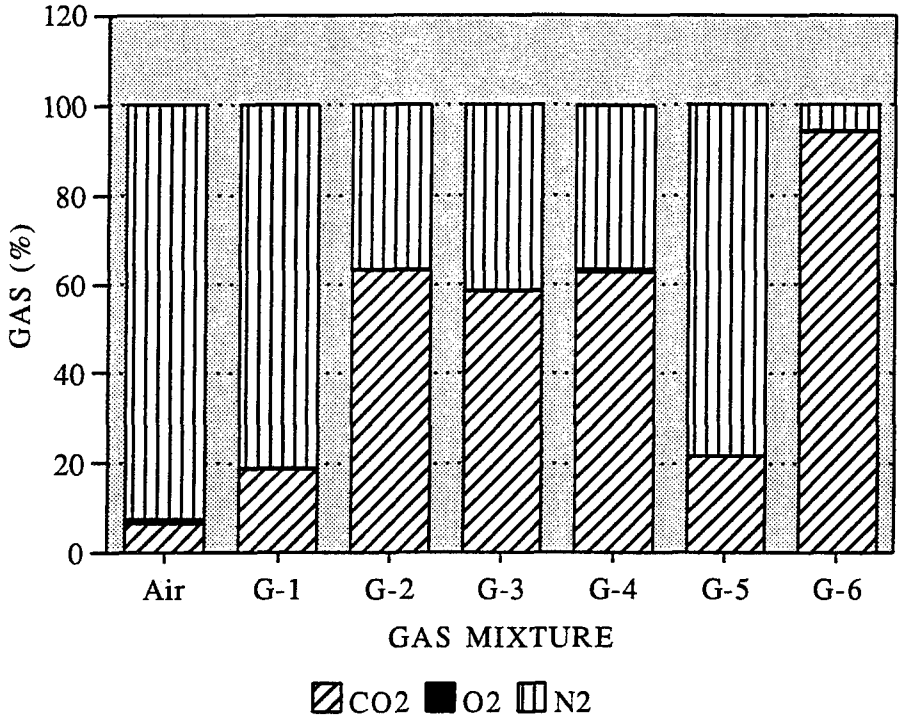

Figure A-4c. Natick MAP hamburgers week 4 gas composition

Figure A-4a. shows the variation in gas volume between the MAP pouch hamburgers within the 6 different test gas mixtures.

Figures A-4b and 4c show the gas compositions of the MAP pouch hamburgers packed in control air and the 6 test gas samples at 0 week and after 4 weeks storage respectively.

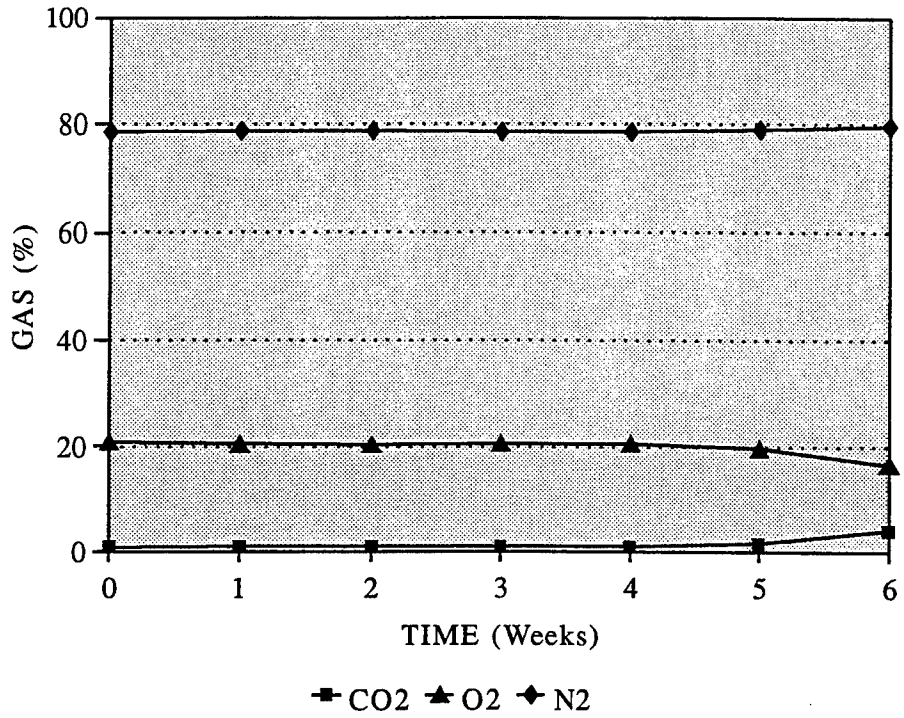

Figure A-5a. Natick MAP eggs air (held) gas composition

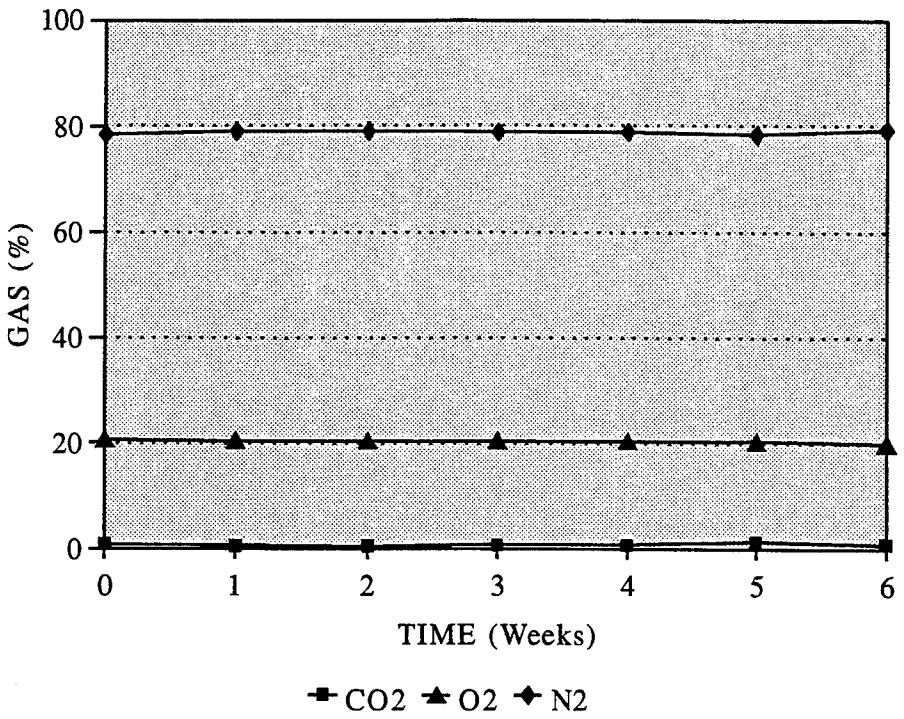

Figure A-5b. Natick MAP eggs air (random) gas composition

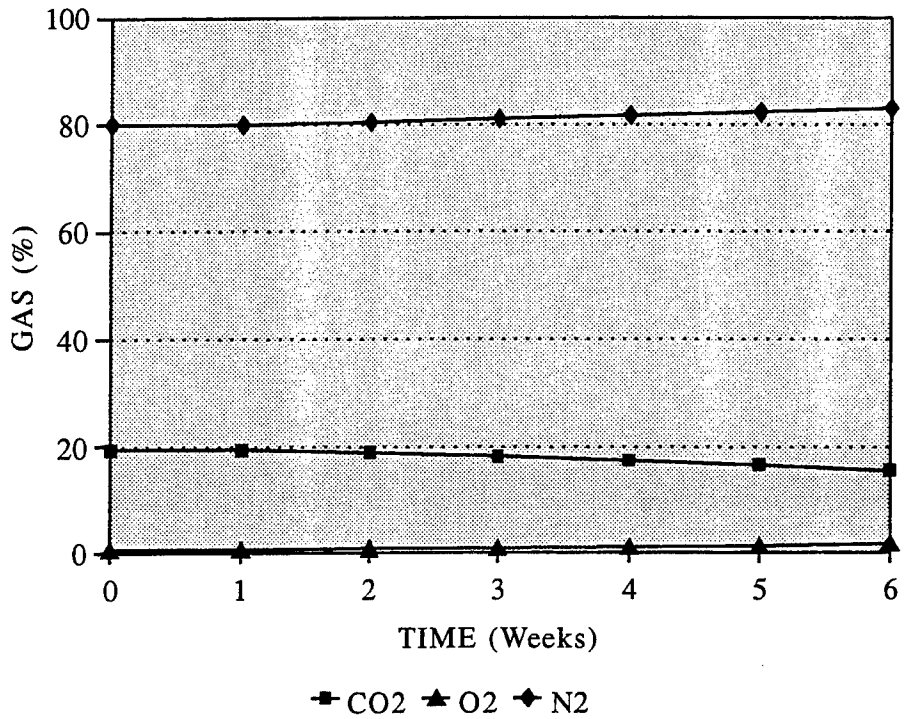

Figure A-5c. Natick MAP eggs gas (held) composition

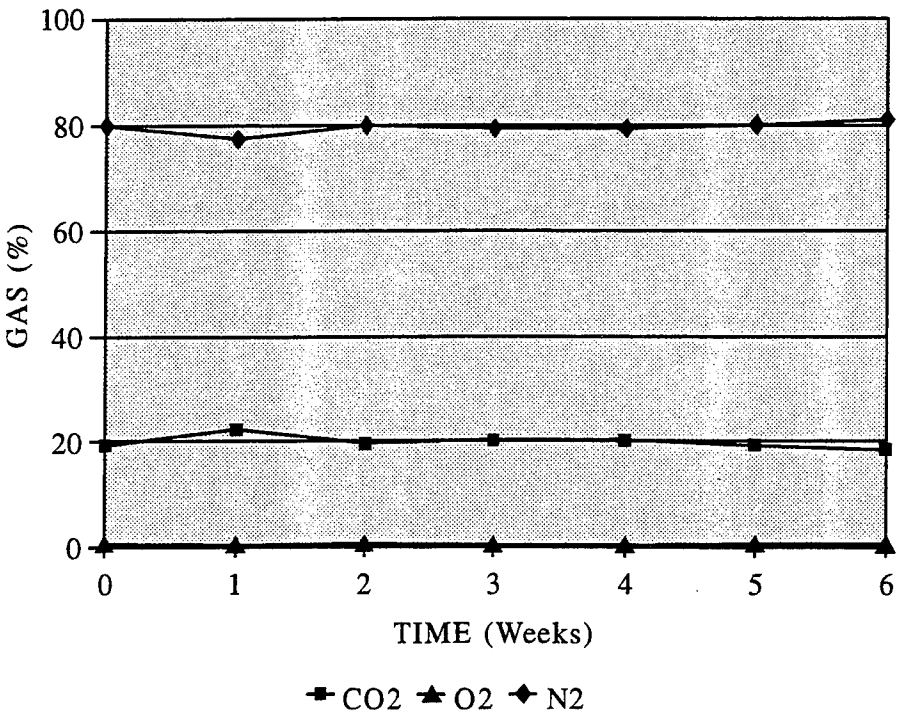

Figure A-5d. Natick MAP eggs gas (random) composition

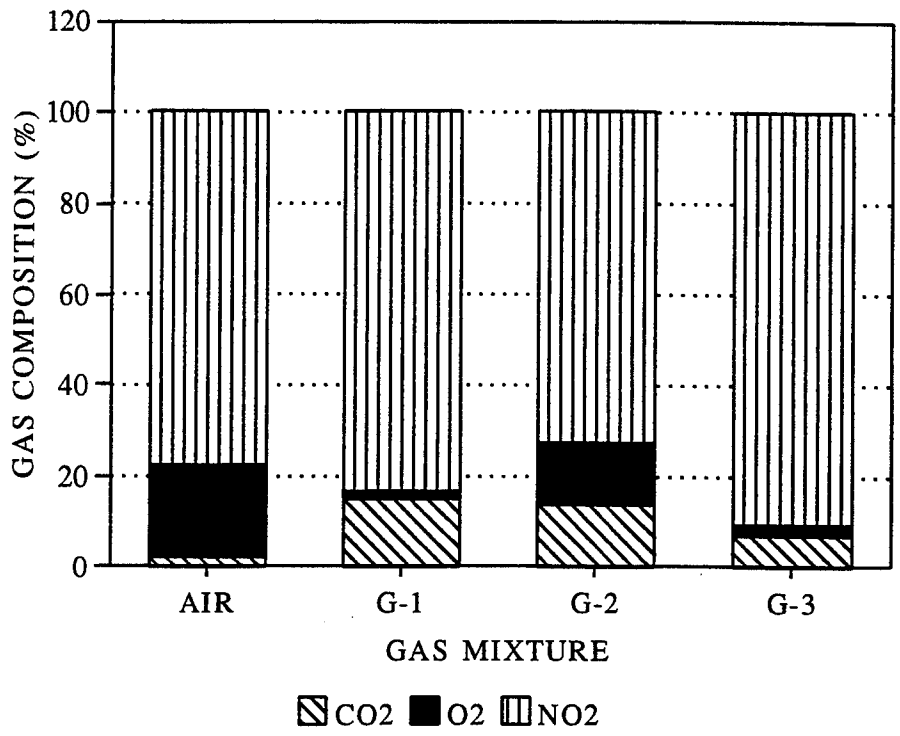

Figure A-6a. Deluca's single serve MAP eggs week 1 gas composition

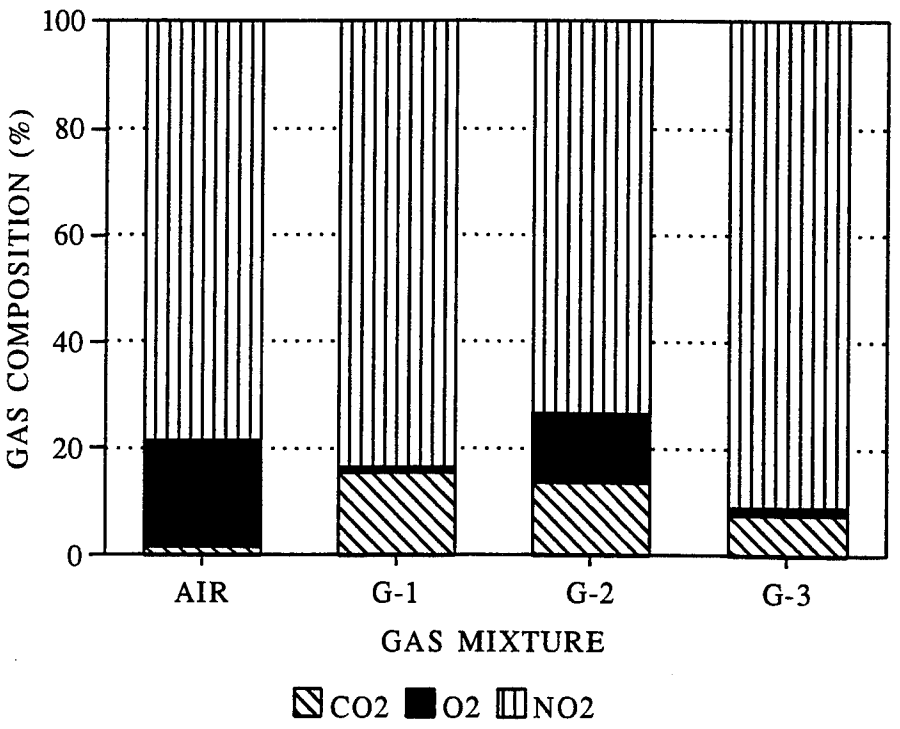

Figure A-6b. Deluca's single serve MAP eggs week 4 gas composition

TABLE B-1. Natick MAP chicken gas analysis data.
Percent gas for each gas test mixture at weekly intervals. *

TIME	PERCENT GAS		
Gas 1 (40% CO2/ 2% O2/ 58% N2)			
	CO2	O2	N2
0WK	24 a	2.97 c	73.03 a
1WK	24.3 a	1.03 b	74.63 bc
2WK	24.6 ab	0.33 a	75.07 c
3WK	24.77 abc	0.2 a	75.03 c
4WK	25.8 bc	0.13 a	74.07 abc
5WK	26.03 c	0.2 a	73.77 ab
Gas 2 (40% CO2/ 5% O2/ 55% N2)			
	CO2	O2	N2
0WK	21.67 a	5.9 a	72.4 a
1WK	21.33 a	4.2 a	74.5 a
2WK	20.9 a	3.83 a	75.27 a
3WK	21.63 a	2.83 a	75.5 a
4WK	23.23 a	1 a	75.77 a
5WK	24.07 a	0.47 a	75.47 a
Gas 3 (40% CO2/ 60% N2)			
	CO2	O2	N2
0WK	25.07 abc	0.2 ab	74.7 ab
1WK	24.73 ab	0.17 ab	75.1 b
2WK	24.6 a	0.23 b	75.17 b
3WK	25.27 bcd	0.17 ab	74.57 ab
4WK	25.6 cd	0.13 a	74.27 a
5WK	25.7 d	0.2 ab	74.13 a
Gas 4 (60% CO2/ 40% N2)			
	CO2	O2	N2
0WK	41.9 a	0.4 a	57.67 a
1WK	40.93 a	0.43 a	58.63 a
2WK	41 a	0.17 a	58.8 a
3WK	41.57 a	0.23 a	58.2 a
4WK	42.07 a	0.13 a	57.77 a
5WK	42.2 a	0.2 a	57.6 a
Air (2% CO2/ 18% O2/ 80% N2)			
	CO2	O2	N2
0WK	0.93 a	17.67 d	81.43 a
1WK	1.7 a	13.5 c	84.83 b
2WK	3.13 b	8.93 b	87.97 c
3WK	7.67 c	1.63 a	90.7 d
4WK	8.07 c	1.2 a	90.73 d
5WK	8.6 c	1.2 a	90.2 d

* Means; N = 3; significant differences indicated by letters ($p<0.05$)

TABLE B-2a. Trio's MAP chicken gas analysis data.
Percent gas for each gas test mixture at weekly intervals. *

TIME	PERCENT GAS		
GAS 1 (40% CO2/.5% O2/ 60% N2)			
	CO2	O2	N2
2WK	14.27	0.3	85.43
3WK	15.1	0.2	84.7
4WK	14.6	0.3	85.1
GAS 2 (40% CO2/ 2.5% O2/ 60% N2)			
	CO2	O2	N2
2WK	12.7	0.65	86.65
3WK	13.4	0.4	86.25
4WK	13.25	0.25	86.5
GAS 3 (40% CO2/ .35% O2/ 60% N2)			
	CO2	O2	N2
2WK	19.4	0.25	80.35
3WK	19.6	0.3	80.1
4WK	19.05	0.25	80.75

* Means; N=3 for gas 1, N=2 for gas 2 & 3;

TABLE B-2b. Trio's MAP chicken microbiological data.
Analysis done two weeks after packaging.

	AEROBIC PLATE COUNTS CFU/g		ANAEROBIC PLATE COUNTS CFU/g
GAS 1	log(6)4.8		log(6)5.05
GAS 2	log(6)1.2		log(6)1.41
GAS 3	log(6)1.23		log(6)1.275
	E. coli	Salmonella	
GAS 1	neg	neg	
GAS 2	neg	neg	
GAS 3	neg	neg	

* Means; N=4

TABLE B-3a. MAP TTI sensory testing guidelines.

Appearance:		Note changes in color, dryness or moisture collection. Visible signs of mold.
Odor	:	Note off odor (due to oxidation, carbon dioxide, etc.)
Flavor	:	Note off flavor (due to oxidation, rancidity, carbon dioxide, etc.) We will collectively decide if there is an off flavor, describe the flavor, and compared to the control what we think is acceptable for this product. We will also try to note the first signs of offness.
Texture	:	Note dryness, moisture migration, and toughness/ tenderness. The control will be frozen so the texture may not be comparable.
Overall	:	Is it still acceptable?

TABLE B-3b. Sensory evaluation form.

TESTER (1-18)

PRODUCT (20-45)

INSTRUCTIONS: Please indicate number for quality scores in the box and make comments in the remaining space. Disregard the small numbers on this form; they are for data processing.

REJECT				BORDERLINE	ACCEPT			
Extremely Poor	Very Poor	Poor	Below Fair Above Poor	Fair	Below Good Above Fair	Good	Very Good	Excellent
1	2	3	4	5	6	7	8	9

SAMPLE (47-49)	APPEARANCE (55)	ODOR (61)	FLAVOR (67)	TEXTURE (73)	OVERALL QUALITY (79)

STSNL Form 964
1 Jul 74

FOOD QUALITY EVALUATION

EDITION OF 1 MAY 74 WILL BE USED UNTIL EXHAUSTED.

TABLE B-3c. MAP TTI testing schedule.
Letters indicate which test was done.

-	Initial abcd
-	Frozen Control a 1 week refrigerated abcd
-	Frozen Control a 1 week refrigerated abused to 70°F for 4 hours abc 1 week refrigerated abused to 100°F for 4 hours abc/e
-	Frozen Control a 2 week refrigerated abcd/e abused 70°F 1 week abc abused 100°F 1 week abc/f
-	3 week refrigerated cd abused 70°F 2 week a/g
-	4 week refrigerated a/g abused 70°F 3 week a/g

a: sensory
b: micro
c: TTI
d: TTI noneventful
e: lasagna not sensory tested due to unacceptable micro
f: chicken nor lasagna not sensory tested due to unacceptable micro
g: appearance and odor only for sensory test

TABLE B-3d. MAP TTI chicken and lasagna sensory data. *

A. CHICKEN SENSORY DATA

	APPEARANCE	ODOR	FLAVOR	TEXTURE	OVERALL
CONTROL	6.3 ab	7.0 a	6.3 a	6.7 a	6.5 a
1 WEEK	6.8 b	6.3 a	6.2 a	6.3 a	6.2 a
1 WEEK ABUSE 70°F	6.5 ab	5.8 a	6.0 a	6.5 a	6.0 a
1 WEEK ABUSE 100°F	6.5 ab	6.2 a	6.2 a	6.3 a	6.0 a
2 WEEK	6.0 a	5.8 a	5.5 a	6.7 a	5.5 a
2 WEEK ABUSE 70°F	6.3 ab	5.8 a	5.8 a	6.0 a	5.8 a

B. LASAGNA SENSORY DATA

	APPEARANCE	ODOR	FLAVOR	TEXTURE	OVERALL
CONTROL	6.7 bc	6.5 a	6.5 a	6.7 a	6.3 a
1 WEEK	7.0 c	6.5 a	6.2 a	6.7 a	6.3 a
1 WEEK ABUSE 70°F	6.2 ab	6.0 a	5.7 a	6.3 a	5.7 a
2 WEEK	5.8 a	6.3 a	6.2 a	6.3 a	6.2 a
2 WEEK ABUSE 70°F	6.8 bc	5.8 a	6.0 a	6.3 a	5.8 a

* Means; N=6 letters indicate significant differences at ($p<0.05$).

TABLE B-3e. MAP TTI chicken and lasagna microbiological analysis data. *

	CHICKEN		LASAGNA	
	Log	CFU/g	Log	CFU/g
CONTROL	0.00 a	<10	0.00 a	<10
1 WEEK	0.00 a	<10	0.00 a	<10
ABUSE @70°F	0.00 a	<10	1.59 ab	448
ABUSE @100°F	1.19 a	30	3.55 bc	208,000
2 WEEK	0.00 a	<10	4.98 c	228,000
2 WEEK ABUSE @70°F	0.00 a	<10	1.73 abc	53,000
2 WEEK ABUSE @100°F	2.64 a	28.6×10^6		

* Means; N=3 letters indicate significant differences at ($p<0.05$).

TABLE B-3f. MAP TTI chicken and lasagna pH data. *

	LASAGNA	CHICKEN
INITIAL	5.2	6.5
WEEK 1	5.47	6.41
ABUSE 70 F	5.55	6.21
ABUSE 100 F	5.37	6.32
WEEK 2	5.15	6.61
WK 2 ABUSE 70 F	5.3	6.23
WK 2 ABUSE 100 F	-	6.5

* Means: N=6

TABLE B-3g. MAP TTI chicken and lasagna no abuse reflectance data. Averages of reflectance readings. *

A. CHICKEN REFLECTANCE DATA

	35°F STORAGE WITHOUT ABUSE		
	LABEL #60	LABEL #67	LABEL #76
INITIAL	91.50 a	67.00 a	75.75 a
1 WEEK	79.25 b	40.62 b	57.00 b
2 WEEK	66.00 c	25.00 c	43.50 c
3 WEEK	50.50 d	00.00 d	00.00 d

B. LASAGNA REFLECTANCE DATA

	35°F STORAGE WITHOUT ABUSE		
	LABEL #60	LABEL #67	LABEL #76
INITIAL	90.50 a	64.50 a	77.25 a
1 WEEK	83.25 b	39.50 b	58.50 b
2 WEEK	68.00 c	23.12 c	40.25 c
3 WEEK	49.00 d	00.00 d	00.00 d

* Means; N=4 letters indicate significant differences at ($p<0.05$).

TABLE B-3h. MAP TTI chicken and lasagna abuse 70 F reflectance data. Averages of reflectance readings. *

A. CHICKEN REFLECTANCE DATA

	35°F STORAGE WITH 4 HOURS ABUSE AT 70°F		
	LABEL #60	LABEL #67	LABEL #76
INITIAL	93.00 a	67.50 a	77.50 a
1 WEEK	81.00 b	37.75 b	59.50 b
ABUSE	80.75 b	38.50 b	59.00 b
2 WEEK ABUSE	66.75 c	21.00 c	38.50 c
3 WEEK ABUSE	46.25 d	00.00 d	00.00 d

B. LASAGNA REFLECTANCE DATA

	35°F STORAGE WITH 4 HOURS ABUSE AT 70°F		
	LABEL #60	LABEL #67	LABEL #76
INITIAL	92.50 a	65.50 a	76.50 a
1 WEEK	79.75 b	34.25 b	54.00 b
ABUSE	79.50 b	33.75 b	29.00 c
2 WEEK ABUSE	56.75 c	17.25 c	29.00 c
3 WEEK ABUSE	36.00 d	00.00 d	00.00 d

* Means; N=4 letters indicate significant differences at ($p<0.05$).

TABLE B-3i. MAP TTI chicken and lasagna abuse 100 F reflectance data. Averages of reflectance readings. *

A. CHICKEN REFLECTANCE DATA

	35°F STORAGE WITH 4 HOURS ABUSE AT 100°F		
	LABEL #60	LABEL #67	LABEL #76
INITIAL	90.75 a	64.75 a	76.00 a
1 WEEK	81.25 b	37.37 b	57.00 b
ABUSE	68.50 c	27.25 c	34.75 c
2 WEEK ABUSE	52.25 d	7.12 d	23.00 d
3 WEEK ABUSE	36.00 e	00.00 e	00.00 e

B. LASAGNA REFLECTANCE DATA

	35°F STORAGE WITH 4 HOURS ABUSE AT 100°F		
	LABEL #60	LABEL #67	LABEL #76
INITIAL	91.00 a	64.75 a	76.00 a
1 WEEK	83.75 a	41.00 b	58.75 b
ABUSE	70.75 b	24.75 c	17.00 c
2 WEEK ABUSE	48.75 c	13.00 d	00.00 d
3 WEEK ABUSE	30.75 d	00.00 e	00.00 d

* Means; N=4 letters indicate significant differences at ($p<0.05$).

TABLE B-3j. MAP TTI chicken and lasagna slope and correlation coefficients for all label reflectance data.

A. LASAGNA SLOPE DATA

LABEL	SLOPE	CORRELATION COEFFICIENT
NO ABUSE		
#60	-13.975	0.9496
#67	-20.988	0.9908
#76	-25	0.9461
ABUSE 70 F		
#60	-13.575	0.8935
#67	-14.8	0.9218
#76	-17.8	0.9375
ABUSE 100 F		
#60	-15.55	0.9164
#67	-15.75	0.9645
#76	-21.075	0.9039

B. CHICKEN SLOPE DATA

LABEL	SLOPE	CORRELATION COEFFICIENT
NO ABUSE		
#60	-13.625	0.9593
#67	-21.6625	0.9858
#76	-24.075	0.9107
ABUSE 70 F		
#60	-10.775	0.9009
#67	-15.175	0.9273
#76	-17.6	0.8805
ABUSE 100 F		
#60	-13.85	0.976
#67	-15.975	0.9426
#76	-18.6	0.988

TABLE B-4a. Natick MAP pouch hamburgers water displacement data or gas volume (ml).

POUCH #	GAS 1	GAS 2	GAS 3	GAS 4	GAS 5	GAS 6	AIR
1	155	152	149	176	238	85	225
2	160	158	155	217	245	315	258
3	250	160	163	225	361	333	280
4	293	165	175	233	465	617	283
5	305	165	228	266	627	658	
6	317	203	239	668		691	
7	324	213					
8	335	215					
9	345	217					
10	350	221					
11	402	224					
12	634	224					

TABLE B-4b. Natick MAP pouch hamburgers gas analysis data.
Percent gas for each gas test mixture at 1 and 4 weeks. **

	GAS MIXTURE	CO2	O2		N2
A. WEEK 0					
AIR		0.3	19.4		80.25
GAS 1	(25% CO2/ 75% N2)	18.45	1.25		80.35
GAS 2	(75% CO2/ 25% N2)	61.85	0.25		37.9
GAS 3	(73% CO2/ 2% O2/ 25% N2)	66.05	1.1		32.85
GAS 4	(70% CO2/ 5% O2/ 25% N2)	54.15	8		37.9
GAS 5	(25% CO2/ 2% O2/ 73% N2)	19.6	3.2		77.2
GAS 6	(97% CO2/ 3% N2)	89.6	5.2		5.2
B. WEEK 4					
AIR		6.4	1.2		92.4
GAS 1	(25% CO2/ 75% N2)	18.85	0.2		80.95
GAS 2	(75% CO2/ 25% N2)	63.25	0.1		36.7
GAS 3	(73% CO2/ 2% O2/ 25% N2)	58.4	0.2	*	41.4
GAS 4	(70% CO2/ 5% O2/ 25% N2)	62.75	0.55	*	36.6
GAS 5	(25% CO2/ 2% O2/ 73% N2)	21.65	0.1		78.3
GAS 6	(97% CO2/ 3% N2)	94.1	0.15	*	5.85

** Means; N=2 * indicates significant differences at ($p<0.05$).

TABLE B-4c. Natick MAP pouch hamburgers microbiological analysis data. *

A. INITIAL STATUS

RAW 3,950-111,000 CFU/g COOKED 5-<10 CFU/g

GAS MIXTURE			AEROBIC		ANAEROBIC	
B. WEEK 0			CFU/g		CFU/g	
AIR			<10		NA	
GAS 1	(25% CO2/ 75% N2)		NA		<10	
GAS 2	(75% CO2/ 25% N2)		NA		<10	
GAS 3	(73% CO2/ 2% O2/ 25% N2)		10		NA	
GAS 4	(70% CO2/ 5% O2/ 25% N2)		<10		NA	
GAS 5	(25% CO2/ 2% O2/ 73% N2)		<10		NA	
GAS 6	(97% CO2/ 3% N2)		NA		<10	
C. WEEK 4			CFU/g	Log	CFU/g	Log
AIR		**	135	3.115	NA	
GAS 1	(25% CO2/ 75% N2)		NA		0	0
GAS 2	(75% CO2/ 25% N2)		NA		14,611	1.935
GAS 3	(73% CO2/ 2% O2/ 25% N2)		7	0.35	0	0
GAS 4	(70% CO2/ 5% O2/ 25% N2)		303	0.77	434	0.985
GAS 5	(25% CO2/ 2% O2/ 73% N2)		33,501	1.2825	33,376	1.2825
GAS 6	(97% CO2/ 3% N2)		NA		0	0

* Means; N=6 ** indicates significant differences at ($p<0.05$).

TABLE B-5a. Natick MAP eggs gas analysis data. Percent gas for each control air and test gas when held and randomly withdrawn. *

A. Control AIR eggs HELD over time

	CO2		O2		N2	
0WK	0.8	a	20.6	a	78.6	a
1WK	0.9	a	20.35	a	78.75	a
2WK	0.95	a	20.3	a	78.75	a
3WK	0.95	a	20.45	a	78.6	a
4WK	0.95	a	20.4	a	78.65	a
5WK	1.55	a	19.5	a	78.95	a
6WK	4.1	a	16.4	a	79.6	a

B. Control AIR eggs RANDOMLY withdrawn over time

	CO2		O2		N2	
0WK	0.8	ab	20.6	b	78.6	a
1WK	0.45	a	20.4	b	79.15	bc
2WK	0.4	a	20.35	b	79.25	bc
3WK	0.7	ab	20.4	b	78.9	ab
4WK	0.65	ab	20.35	b	78.9	ab
5WK	1.35	b	20.2	ab	78.45	a
6WK	0.8	ab	19.8	a	79.4	c

C. Test gas (25% CO2/ 75% N2) eggs HELD over time

	CO2		O2		N2	
0WK	19.35	e	0.6	a	80.05	a
1WK	19.3	e	0.6	a	80.1	a
2WK	18.85	de	0.7	a	80.45	ab
3WK	18.1	cd	0.85	ab	81.05	bc
4WK	17.3	bc	1.05	bc	81.65	cd
5WK	16.55	ab	1.2	c	82.25	de
6WK	15.45	a	1.55	d	83	e

D. Test gas (25% CO2/ 75% N2) eggs RANDOMLY withdrawn over time

	CO2		O2		N2	
0WK	19.35	a	0.6		80.05	b
1WK	22.3	b	0.25		77.45	a
2WK	19.6	a	0.5		79.95	b
3WK	20.2	ab	0.4		79.4	ab
4WK	20.2	ab	0.35		79.45	ab
5WK	19.35	a	0.55		80.1	b
6WK	18.45	a	0.45		81.1	b

* Means; N=2 letters indicate significant differences at ($p<0.05$).

TABLE B-5b. Oxygen transmission rate of packaging films.

MATERIAL	TRANSMISSION RATE (cc/m sq/day)
Curlam 8057K *	3.78
(bottom)	3.89
Curform 7748 *	2.51
(lidstock)	
Printpack	3.42
(bottom)	
Printpack LS	2.64
(lidstock)	2.73

* Packaging material chosen for the MAP egg study.

TABLE B-5c. Natick MAP eggs chemical analysis.

PROTEIN	16.195	%
FAT	14.42	%
MOISTURE	66.45	%
ASH	1.285	%
pH	7.97	
Aw	0.975	

TABLE B-5d. Natick MAP eggs microbiological analysis and pH over a 6 week testing period. *

	Control Air		Test Gas	
	AEROBIC CFU/g	pH	ANAEROBIC CFU/g	pH
0 WEEK	<10	8.22	<10	7.92
1 WEEK	<10	8.38	<10	8.205
2 WEEK	<10	8.41	<10	8.105
3 WEEK	805	8.29	<10	8.225
4 WEEK	1950	8.21	<10	7.815
5 WEEK	370	8.235	<10	7.9
6 WEEK	120	8.27	<10	8.09

* Means: N=4

TABLE B-6a. Oxygen transmission rate of packaging material used for Deluca's MAP eggs study.

PACKAGING MATERIAL	TRANSMISSION RATE (cc/m sq/day)
Deluca Multi-Serve Bag	3.172
	3.442
Deluca Single Serve Bottom	Fail *
	Fail
Deluca Single Serve Lid	2.553
	3.122
	2.434
	2.947

* Failure may be due to instrument malfunction or irregular sample.

TABLE B-6b. Deluca's MAP eggs gas analysis data.
Percent gas for each test gas mixture for single serve after 1 and 4 weeks storage and multi-serve after 1 week storage. **

A. SINGLE SERVE EGGS

	1 WEEK	CO2	O2	N2
AIR		2.1	20.4	77.52
GAS 1	40% CO2/ 60% N2	15.25	1.55	83.25
GAS 2	40% CO2/ 10% O2/ 50% N2	13.98	13.52	72.52
GAS 3	25% CO2/ 75% N2	7.18	2.38	90.45

	4 WEEK	CO2	O2	N2
AIR		1.62	19.78 *	78.58
GAS 1	40% CO2/ 60% N2	15.38	1.1	83.52
GAS 2	40% CO2/ 10% O2/ 50% N2	13.48	13.05	73.45 *
GAS 3	25% CO2/ 75% N2	7.6	1.42 *	90.98

B. MULTISERVE EGGS

	1 WEEK	CO2	O2	N2
AIR		1.075	19.925	79
GAS 1	40% CO2/ 60% N2	1.775	14.725	83.55
GAS 2	40% CO2/ 10% O2/ 50% N2	1.575	14.675	83.5
GAS 3	25% CO2/ 75% N2	1.275	14.35	83.325

** Means; N=4 * indicates significant differences at ($p<0.05$).

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